THE TAKIN AND MUSKOX:

A MOLECULAR AND ECOLOGICAL EVALUATION OF RELATIONSHIP

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

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Fairbanks, Alaska

May 1995

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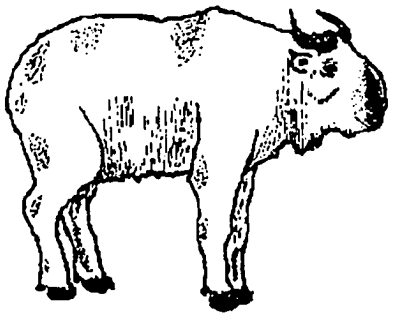
THE TAKIN AND MUSKOX



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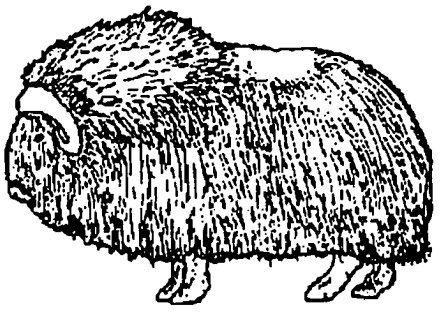

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ABSTRACT

This research clarifies the classification of and relationship between the takin (*Budorcas taxicolor*) and muskox (*Ovibos moschatus*). Although both are social ungulates of similar body size, takins live in dense mountainous habitats at temperate latitudes in Asia, and muskoxen live in open arctic habitats in Alaska, Canada and Greenland. Morphological, paleontological and chromosome comparisons have supported a close relationship between these species and their classification within the tribe Ovibovini in the subfamily Caprinae. Previous studies, however, have not defined the genetic relationship of the takin and muskox. This project used ecological and molecular comparisons to evaluate these proposed relationships.

Ecological studies in Shaanxi, China showed takins are generalists in their use of habitat and forage, but live in dense habitat in groups, ostensibly to avoid predation. Likewise, muskoxen live in groups and are generalists, but inhabit open landscapes. Some ecological and behavioral similarities appear to support the hypothesis of close relationship. In contrast, molecular studies using cytochrome *b* sequences from mitochondrial DNA (mtDNA) clearly separate the takin and muskox into distinct clades. Takins are more closely related to sheep (*Ovis spp.*) and muskoxen to the Asian goral (*Nemorhaedus caudatus*). Similarities between the takin and muskox appear due to convergent evolution as an adaptation to large body size. A broader comparison of cytochrome *b* sequences from 11 species of Caprinae supported the separation of takins and muskoxen. Unequal rates of evolution among the species precluded complete resolution of Caprinae relationships.

To define differences between muskox subspecies and populations, sequences of the highly variable control region of mtDNA from 37 muskoxen were compared. Delineation of muskox subspecies is a critical issue due to the potential for interbreeding of indigenous Canadian (*O. m. moschatus*) and introduced Alaskan (*O. m. wardi*) populations of muskoxen as range expansion occurs. Variability between these populations was so low differences could not be detected with this comparison. I suggest a history of genetic bottlenecks has reduced genetic variability of muskoxen to low levels and neither populations nor subspecies can be defined from control region sequences.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

Over the years, many organizations have contributed funding for this project and I am grateful for their support. The Boone and Crockett Club provided funding for the DNA analysis. The Explorers Club, Institute of Arctic Biology, University of Alaska Fairbanks and Shaanxi Institute of Zoology, Xian, China all contributed funding for the field work in China. A TOTE Dissertation Year Fellowship, UAF Foundation Scholarships, the Institute of Arctic Biology and Program in Biochemistry and Molecular Biology, UAF all contributed support during my last years of analyzing and writing.

I particularly would like to acknowledge Bob White who encouraged me to pursue the takin research back when I was a humble muskox farmer and who helped to facilitate the entire project. Bob served as the chairman of my graduate advisory committee. My other committee members, Gerry Shields, Dave Klein, Terry Bowyer and Lyle Renecker all provided support, encouragement and patience over the years.

To arrange for a foreigner to conduct field research in China is not always easy, but I had extremely good cooperation with the Chinese and I thank the many organizations and people who helped with the study. Logistical support was provided by the Shaanxi Institute of Zoology and orchestrated by Zhu Xunmei and Han Yiping with the approval of the Chinese Ministry of Forestry, the Conservation Bureau of the Ministry of Forestry and the Wildlife Station of the Shaanxi Forestry Department. Wang Jianbin, Xiao Hong, Zhang Chongyi, Xiao Cheng, Xiao Zhang and Jiang Mian Chong helped with the field surveys. I especially would like to recognize the contribution of Wu Jiayan to the success of this project.

Tissue samples for the DNA analyses were provided by many people and organizations and I appreciate their contributions. They include: Anne Gunn, Bruce McLean and Paul Fraser from the Department of Renewable Resources, Government of the Northwest Territories; Greenland Environmental Research Institute, Copenhagen; Large Animal Research Station, UAF; Musk Ox Development Corporation, Palmer AK; Shaanxi Institute of Zoology; Nick Reindl, Minnesota Zoo; Oliver Ryder, San Diego Zoo; Curtis Strobeck, University of Alberta and Greg Finstad, Fairbanks AK.

I also would like to thank some of the many other people who have helped me: Judy Reed Gust for teaching me lab techniques, Bill Hauer for help with computer glitches, Joe Cook and Mark Lindberg for DNA analysis discussions, Mitch Wagener and Jay Gulledge for technical help with slide preparation, the Animal Quarters crew for their own kind of support and my wonderful friends who made sure I had fun. Finally, I would like to acknowledge my family who have always provided encouraging support even if they did not understand what I was doing or why it was taking so long!

LIST OF MANUSCRIPTS

Chapters of this thesis have been written as independent manuscripts to be submitted to peer-reviewed journals for publication. Some have been submitted already, others are in the final stages of preparation for submission. These papers are as follows:

Muskox (*Ovibos moschatus*).

Pamela Groves, Institute of Arctic Biology, University of Alaska Fairbanks. C.I.C. Caprinae Atlas (In press).

Takin (*Budorcas taxicolor*).

Pamela Groves, Institute of Arctic Biology, University of Alaska Fairbanks. C.I.C. Caprinae Atlas (In press).

A Social Ungulate in Dense Habitat: Group Size and Habitat Use of the Golden Takin.

Pamela Groves, Institute of Arctic Biology, University of Alaska Fairbanks and Wu Jiayan, Shaanxi Institute of Zoology, Xian, China. (In prep.).

Convergent Evolution of the Asian Takin and Arctic Muskox.

Pamela Groves and Gerald F. Shields, Institute of Arctic Biology, University of Alaska Fairbanks. (Submitted to Journal of Mammalogy).

Phylogenetics of the Caprinae Based on Cytochrome *b* Sequence.

Pamela Groves and Gerald F. Shields, Institute of Arctic Biology, University of Alaska Fairbanks. (Submitted to Molecular Phylogenetics and Evolution).

Muskoxen Have Little Intraspecific Variation Based on Sequences of Mitochondrial DNA.

Pamela Groves, Institute of Arctic Biology, University of Alaska Fairbanks. (In prep, for submission to Canadian Journal of Zoology).

The Case for Convergence: the Takin and Muskox

Pamela Groves, Institute of Arctic Biology, University of Alaska Fairbanks. (In prep.).

INTRODUCTION

This thesis is the result of my involvement with two species from two different continents that were thought to be related: the Asian takin (*Budorcas taxicolor*) and the arctic muskox (*Ovibos moschatus*). My interest in muskoxen began in 1979 when I began a career managing a herd of captive muskox in Unalakleet on the west coast of Alaska. After four years with the muskoxen, I moved to China where I began to make inquiries about researching takins. Eventually, those inquiries bore fruit and I was invited to study wild takins in Shaanxi Province. Thus, I embarked upon this project to gather information I thought would support and confirm the relationship of the takin and muskox.

The muskox is a large ungulate with long, brown hair and white out-curved horns. Indigenous populations of muskoxen are distributed across arctic and subarctic regions of Canada and Greenland. Introduced populations of muskoxen have been established in Alaska, Russia, Norway and Sweden, west Greenland and northern Quebec (CHAPTER 1).

The takin which is less well known than the muskox is a threatened species with the same protected status as the giant panda (*Ailuropoda melanoleuca*) within China. The takin is about the same size as the muskox, but has short hair ranging in color from golden-yellow to dark brown and has black upward-curved horns. Takins are distributed across mountainous regions of Asia at temperate latitudes (CHAPTER 1).

Classification of these two species has generated much debate. Initially each was thought to be closer to cattle than to sheep and goats (Hodgson, 1850; Allen, 1913). Close relationship between the takin and muskox was proposed as early as 1850 when the takin was first described (Hodgson, 1850) and was based solely on morphological similarities. Subsequent morphological, paleontological and chromosome comparisons (Lander, 1919; Bogart and Benirschke, 1975; Harington, 1989; Pasitschniak-Arts et al., 1994) have been used to further support close relationship between the species. Simpson (1945) classified the takin and muskox as the only two extant species within the tribe Ovibovini in the subfamily Caprinae, a classification that has been widely accepted. No previous study, however, has been

able to support definitively the relationship of these species and debate about it has continued (Lent, 1988).

Takins tend to inhabit densely vegetated areas within mountain ranges. Due to the remoteness of these regions and the rugged terrain, takin ecology has not been extensively studied. The first objective of my project, therefore, was to observe takin ecology for similarities with muskox ecology which might support their close relationship. I hypothesized that similarities in ecology despite different habitats might indicate a common evolutionary background of the species.

I spent about six months in 1988 and 1990 studying takin ecology in the Qinling Mountains of Shaanxi Province. The dense vegetation made observing takins extremely difficult, but I was able to collect data on group size in relation to habitat, habitat use and selection of feeding habitats (CHAPTER 2).

Although most muskoxen inhabit remote regions of the Arctic, the openness of their habitat has facilitated ecological studies and much information on muskox ecology is available (Gray, 1987; Lent, 1988). I was able to supplement published muskox studies with my own observations of muskoxen made on Banks Island, NWT, Canada during the summers of 1989 and 1990.

Recent technological advances have made the determination of mitochondrial DNA (mtDNA) sequences a practical tool for evaluating controversial and unresolved aspects of phylogeny. MtDNA has proved useful for phylogenetic reconstructions because it is maternally inherited with no recombination which facilitates tracing lineages back in time and because of its more rapid rate of evolution relative to nuclear DNA (Brown et al., 1982). The cytochrome *b* gene which is a protein-coding gene is well understood and has a rate of evolution which makes it appropriate for evaluating interspecific relationships (Irwin et al., 1991). The second objective of my project was to use modern molecular techniques to test the hypothesis of close relationship between the takin and muskox by comparing sequences from the cytochrome *b* genes of both species (CHAPTER 3).

To place the relationship of the takin and muskox within a framework, I included representative species from each of the other Caprinae tribes (Simpson, 1945) in this analysis. These species were: saiga (*Saiga tatarica*) from the Saigini, Chinese goral

(*Nemorhaedus caudatus*) from the Rupicaprini and bighorn sheep (*Ovis canadensis*) from the Caprini. The published sequence of domestic cow (*Bos taurus*) was included as an outgroup (Irwin et al., 1991).

The results of this comparison of six species were unexpected and led me to wonder about the broader picture of Caprinae phylogenetics. Thus, the third objective of my project was to investigate the relationships of the diverse Caprinae species by analyzing sequences of the cytochrome *b* gene from 12 species. In addition to the six species above, I included Japanese serow (*Capricornis crispus*), mountain goat (*Oreamnos americanus*), Himalayan tahr (*Hemitragus jemlahicus*), Dall's sheep (*Ovis dalli*), domestic goat (*Capra hircus*) and domestic sheep (*Ovis aries*; CHAPTER 4).

While sequencing the cytochrome *b* gene from 38 muskoxen from diverse populations, I observed less variation than has been reported for this gene in other mammalian species (Shields and Kocher, 1991; Smith and Patton, 1991; Mouchaty et al., 1995). This apparent lack of intraspecific variability stimulated the fourth objective of my project which was to attempt to use mtDNA sequences to define differences between muskox subspecies and populations. For this analysis, I used control region comparisons of 37 muskoxen from eight populations. The control region of mtDNA which is non-coding and therefore the most rapidly evolving region of the molecule, is appropriate for investigating relationships at the intraspecific level (Avise et al., 1987). (CHAPTER 5).

Two subspecies of muskoxen are commonly recognized, *O. m. moschatus* on the mainland of Canada and *O. m. wardi* on the arctic islands and in Greenland. The introduced populations of muskoxen all are *O. m. wardi.* There is a strong possibility of the mixing of the transplanted Alaskan (*O. m. wardi)* and indigenous Canadian (*O. m. moschatus)* muskoxen as both populations expand their ranges. Concern has developed about the interbreeding of these two formerly separate populations because their mixing would be the result of human manipulation. The management implications of the mixing of the two muskox populations make the definition of muskox subspecies a crucial issue at this time.

My research has confirmed some similarities between the takin and muskox and revealed differences as well. In the final chapter of this thesis, I have summarized both

the similarities and differences and attempted to explain how they might have evolved (CHAPTER 6). In the course of all this research, I have maintained my fascination with these species. Both have intriguing adaptations to their respective environments which would be interesting to investigate further.

CHAPTER 1 SPECIES DESCRIPTIONS

MUSKOX (*OVIBOS MOSCHATUS*)

The muskox is a gregarious species indigenous in historical times to arctic regions of North America and Greenland. European fur traders and explorers to North America returned home in the 18th and 19th century with tales of strange buffalo-like animals encountered in the far north. In 1780, the muskox was introduced in the literature as *Bos moschatus* by Zimmerman (1780). The genus *Ovibos*, proposed by Blainville in 1816, gained acceptance by the mid-1850's (Allen, 1913). The muskox is the only extant species within the genus *Ovibos*, although the takin (*Budorcas taxicolor*) is classified with the muskox in the tribe Ovibovini within the subfamily Caprinae (Simpson, 1945).

Muskoxen are large-bodied with a hump at the shoulders and sloping rump, producing a superficial resemblance to bison (*Bison bison*). Legs are short and stocky with broad black hooves well-adapted for walking on hard snow and tundra. A short tail (60 to 140 mm; Tener, 1965) is completely hidden under the hair coat. The short ears (150 mm; Tener, 1965) are hair-covered and, in winter, are obscured by hair of the head and neck. Hair extends down the muzzle to the edge of broad, square lips. Muskoxen, particularly males, have massive heads with protruding orbits. Both males and females possess preorbital glands (Lönnberg, 1900a). The most distinguishing characteristics of the muskox are the cream-colored, out-curved horns and the long brown hair coat.

The horns which originate between the orbits and occipital plane (Lydekker, 1898) grow laterally in young muskoxen. After 2 years, horns begin to curve forward and down. Mature animals possess longitudinally striated horns which curve down, slightly forward and up to a sharp black point forming hooks that are effective weapons for predator defense. The horn bases of females remain separated and covered with white hair. On males, the horn bases grow almost together and thicken to form a

pronounced massive boss. During the mating season when males fight by engaging in series of head-on clashes, the horn boss provides protection for the head (Gray, 1987).

Much of the body of adult muskoxen is covered by long brown guard hairs that extend almost to the ground; individual hairs can exceed 600 mm in length (Allen, 1913; Lent, 1988). Guard hairs on the back of the neck can be 300 mm long (Rowell, 1990) and stand upright accentuating the size of the neck. The hair on the center of the back is short (<150 mm; Allen, 1913) and cream-colored creating an obvious saddle. The legs have short coarse white hair that extends partially over the hooves. Under the guard hair, the entire body of the muskox is covered by a layer of thick soft underwool known by the Eskimo term "qiviut". Qiviut fibers, which average 10 to 16 μm in diameter and 40 to 80 mm in length (von Bergen, 1931; Wilkinson, 1975), provide excellent insulation against the cold of arctic winters. The qiviut is shed annually in spring and regrown during summer. Captive adult muskoxen yield 2.6 to 3.5 kg of qiviut annually (White et al., 1989b).

Due to logistical difficulties, few weights of wild muskoxen have been reported. Both wild and captive muskoxen show seasonal weight variations of up to 30 kg as well as individual variations (Hubert, 1974; White et al., 1989a; Groves, 1992). Animals from the Canadian mainland tend to outweigh those from other locations (Tener, 1965; Lent, 1988). Weights of mature wild males have been reported to range between 191 and 373 kg, those of females may range between 143 and 288 kg (Tener, 1965; Hubert, 1974; Latour, 1987). One captive male weighed 636 kg (Teal, 1970), although in the captive herd at Palmer, Alaska, males tend to weigh between 250 and 368 kg and females between 182 and 318 kg (Groves, unpub. data).

Other measurements of wild male muskoxen include the following ranges: height at shoulder, 1230 to 1725 mm; body length, 1760 to 2460 mm; length of horn, 540 to 744 mm; breadth of horn base, 131 to 325 mm and distance between horn tips, 460 to 750 mm. Measurements of wild females include: height at shoulder, 1230 mm; body length, 1660 to 2091 mm; length of horn 355 to 505 mm; breadth of horn base, 72 to 103 mm and distance between horn tips, 368 to 554 mm (Lydekker, 1898; Allen, 1913; Hone, 1934; Tener, 1965; Latour, 1987).

The muskox has a fundamental chromosome number (FN) of 60 (Tietz and Teal, 1967). The modal diploid number (2n) is 48 with a karyotype of six pairs of submetacentric or metacentric autosomes and 18 pairs of acrocentric autosomes. The X is the largest acrocentric chromosome and the Y is the smallest metacentric element (Heck et al., 1968). The takin also has a FN of 60, but the takin diploid number is 52 (Bogart and Benirschke, 1975). Bogart and Benirschke (1975) propose that Robertsonian fusion of four pairs of acrocentric chromosomes could have yielded the two additional metacentric pairs in the muskox and thus support the proposed close relationship of the two species.

Muskoxen are social animals and are typically observed in groups of 10 to 20 individuals, although some mature bulls may move independently (Smith, 1976; Gray, 1987). The groups frequently try to defend themselves from predators by forming a compact bunch with their rumps pressed together and their heads and horns facing outwards. Primary predators are wolves (*Canis lupus*), but grizzly bears (*Ursus arctos*) and polar bears (*Ursus maritimus*) also have been reported to prey on muskoxen (Gunn and Miller, 1982; Gray, 1987).

The current range of muskoxen is limited to arctic and subarctic regions. The thick layer of qiviut and fat reserves accumulated during summer help them survive extremes of winter. In winter, muskoxen reduce their activity, but continue to forage in regions blown free of snow or with little snow by digging craters through the snow to obtain food (Gray, 1987).

Subspecies

Historically, muskoxen were classified in 2 genera and 5 species until Allen (1913) reduced the confusion by proposing one genus, *Ovibos*, one species, *O. moschatus*, and three subspecies. Based on the lack of statistically significant differences in morphological measurements, Tener (1965) rejected all subspecies of muskoxen. Presently, based on physical characteristics and geographical separation, two subspecies, *O. m. moschatus* and *O. m. wardi*, are commonly recognized by most authors (Rowell, 1990). The author currently is sequencing mitochondrial DNA from diverse muskox populations to attempt to define subspecific differences.

Barren-ground Muskox

Ovibos moschatus moschatus Zimmerman, 1780:86. Type locality near Churchill, Manitoba, Canada.

The first written reports of muskoxen were of *O. m. moschatus* encountered on the western shores of Hudson Bay. These muskoxen tend to be the largest of the species. Their faces are dark with traces of white hair. The horn bases of males are usually broader than those of other muskoxen (Allen, 1913).

This subspecies is limited to arctic regions of mainland Canada north of treeline. While the first specimens of barren-ground muskoxen were reported in northern Manitoba, their numbers dropped dramatically by the turn of the century and their present range is limited to within the Northwest Territories (north of 60° N) where their distribution is spotty (Barr, 1991). Because the Canadian arctic mainland is so vast and remote, delineating the range of muskoxen is extremely difficult. The current range is mostly west of Baker Lake and Chantrey Inlet, north of Great Slave and Great Bear Lakes and east of Colville Lake and Parry Peninsula. Numbers of muskoxen on the Canadian mainland are increasing and the animals appear to be recolonizing historic ranges (Case et al., 1989). Based on surveys conducted between 1986 and 1988, about 18,000 muskoxen are estimated to inhabit the Canadian mainland (Gunn, 1990).

White-faced Muskox

Ovibos moschatus wardi Lydekker, 1900:157. Type locality Clavering Island, Greenland.

Individuals of this subspecies have the distinction of inhabiting the northernmost land on the globe in Greenland (83° N). As the name implies, *O. m. wardi* has a whiter face than *O. m. moschatus.* The amount of white varies widely among individuals and there is a tendency for the saddle area and horns to be lighter in color (Allen, 1913). This subspecies also tends to be smaller than the mainland variety.

The current range of indigenous populations of *O. m. wardi* extends from about 70° N on the east coast of Greenland north along the coast of north Greenland and westward through most of the arctic islands of Canada. With the exception of Baffin Island, muskoxen are found on most of the large islands including: Banks, Victoria,

Prince of Wales, Devon, Somerset, Prince Patrick, Melville, Bathurst, Axel Heiberg and Ellesmere Islands (Barr, 1991).

Many of these populations have fluctuated dramatically within recent history. These fluctuations combined with the remoteness of the range have made defining the exact distribution and populations of *O. m. wardi* extremely difficult. Current estimates of the indigenous Greenland populations are between 9,500 and 12,500 animals (Boertmann et al., 1992). Estimates for the Canadian archipelago muskox populations, based on surveys between 1985 and 1991, are about 87,000 animals (Ferguson and Gauthier, 1992).

Translocated Populations

Muskoxen once had a wider distribution than their present natural range. A number of projects have been undertaken to translocate muskoxen, both to reestablish them in native ranges and to introduce them to other areas of suitable northern habitat.

Muskoxen disappeared from Alaska sometime in the mid-1800's, possibly due to a combination of environmental factors and hunting pressure (Hone, 1934; Burch, 1977). In 1930, 34 muskoxen (*O. m. wardi*) were translocated from east Greenland to Alaska to reestablish the species in Alaska and to investigate the potential of domestication (Bell, 1931). These animals were kept in captivity in Fairbanks for studies until 1935-36 when they were released into the wild on Nunivak Island (Klein, 1988).

Since 1967, several groups of muskoxen have been translocated from Nunivak Island to other areas in Alaska including: Nelson Island, adjacent to Nunivak Island, Seward Peninsula and Cape Thompson in northwest Alaska and northeastern Alaska, in and adjacent to the Arctic National Wildlife Refuge (ANWR). These populations all appear to be well established. Present approximate estimates of these populations are as follows: Nunivak Island, 530; Nelson Island, 230; Seward Peninsula, 800 (R. Kacyon, Alaska Dept. Fish & Game, pers. comm.); Cape Thompson, 130 (S. Machida, Alaska Dept. Fish & Game, pers. comm.); ANWR and surrounding areas, about 600 (P. Reynolds, U.S. Fish & Wildlife Service, pers. comm.). The animals in ANWR have been dispersing both to the west within Alaska and east into the Yukon Territory, Canada. Between 50 and 100 muskoxen may now be in the Yukon (P. Reynolds, pers.

comm.). A captive herd of muskoxen was established from animals captured on Nunivak in 1964-65. This herd, which is raised for qiviut to support a native knitting industry, is located in Palmer, Alaska and presently numbers about 75 animals (Groves, 1992). A second captive herd of muskoxen from Nunivak Island was established for research purposes by the Institute of Arctic Biology, University of Alaska Fairbanks in 1979 and now numbers about 30 animals.

Fossil evidence indicates muskoxen inhabited regions of Siberia (Russia) until about 3000 years ago (Vereschagin, 1959; Klein, 1982). Forty muskoxen from Nunivak Island, Alaska and 10 from Banks Island, Canada (*O. m. wardi*) were translocated to Siberia in 1974-75. The Canadian and 20 Alaskan animals were released on the Taimyr Peninsula and the remaining 20 on Wrangel Island (Uspenski, 1984). In 1987, the Taimyr population was estimated to be 230 animals and the Wrangel Island population to be 70 (Yakushkin, 1989).

Thirteen muskoxen from Ellesmere Island, Canada (*O. m. wardi*) were introduced to the Ungava Peninsula of northern Quebec, Canada, an area with no known previous history of muskoxen, as part of a domestication project in 1967 (Wilkinson and Teal, 1984). Domestication efforts ceased after 5 years and 54 muskoxen were subsequently released in three areas of northern Quebec. By 1986, these populations were estimated to total 350 animals and were increasing rapidly (Klein, 1988; Le Hénaff and Crête, 1989).

Between 1947 and 1953, 27 muskoxen were translocated from northeast Greenland (*O. m. wardi*) to the Dovre Mountains of Norway. This population has remained small, at about 35 animals (Klein, 1988). In 1971, five animals dispersed from Norway into Sweden and established a population that was estimated at 40 individuals in 1985, but apparently now is declining (Lundh, 1984).

Within Greenland, muskoxen have been translocated to fill presumed unoccupied ecological niches, to provide a population reserve of muskoxen, to add to the subsistence food base of native people and to reestablish vanished populations (Vibe, 1967; Klein, 1988). In 1962-65, 27 animals (*O. m. wardi*) were moved from eastern Greenland to the Kangerlussuaq area of western Greenland. This population has increased to an estimated 2,600 animals in 1990 (Boertmann et al., 1992). In

1986, 27 animals from this population were translocated to three areas in the Avanersuaq region of northwest Greenland, an area that historically had muskoxen (Klein, 1988). There has been illegal hunting of these animals and no current estimate of their numbers is available (Boertmann et al., 1992). An additional 15 animals from the Kangerlussuaq population were translocated to the Ivittuut area of southwest Greenland in 1987. By 1990, this population had divided into two groups and totaled 42 animals (Boertmann et al., 1992).

Synonyms

The generic name, *Ovibos*, was proposed by Blainville (1816) to describe both the sheep-like (Ovis) and cattle-like (Bos) characteristics of the animal. The specific name, *moschatus*, was introduced by Zimmerman (1780). Zimmerman based his name on descriptions by Jérémie, a French officer stationed on the west coast of Hudson Bay from 1697 to 1714. Jérémie referred to muskoxen as "Boeufs-musquez" and described them as a kind of cattle smelling strongly of musk (Allen, 1913).

The common name, muskox, may be an anglicized version of the French "boeuf musquez" which literally means musk cattle. The name is a misnomer as muskoxen do not possess musk glands and are not cattle or oxen. Wilkinson (1971) suggested the name may come from the Indian word "muskeg" which describes some of the habitat where the species is found. The Eskimo name for the species is "oomingmak" (various spellings are possible) which means "bearded one" and describes the appearance of mature muskoxen with their long hair.

Status

Within recorded history, muskox populations have been observed to fluctuate dramatically. Extreme declines in muskox populations have resulted in localized die-offs, such as occurred in Alaska, some Canadian islands and northwest Greenland (Hone, 1934; Thing et al., 1984; Barr, 1991). A dramatic decline of muskox numbers occurred in the late 1800's possibly due to a combination of human hunting pressure and environmental factors (Vibe, 1967; Burch, 1977). The species was thought to be in danger of extinction and in an effort to save the species, the Canadian government granted the muskox complete protection from hunting in 1917 (Tener, 1965).

Muskoxen in Canada began to recover from their decline sometime in the early 1900's, although as recently as the 1960's numbers were still low (Tener, 1965). Subsequently, many populations have increased dramatically, particularly those on Banks and Victoria Islands where almost two thirds of Canadian muskoxen now occur (Gunn, 1990; Gunn et al., 1991). With about 100,000 muskoxen in Canada, the government has instituted a system of quotas by region for hunting of muskoxen. These quotas allow for over 6000 muskoxen to be hunted annually (A. Gunn, pers. comm.); although not all of the quotas are filled (Case et al., 1989).

Indigenous muskoxen in Greenland increase during periods of favorable weather and decrease during adverse climatic trends (Vibe, 1967; Thing, 1990). Within the last decade, some populations in northeast Greenland have decreased dramatically (Boertmann et al., 1992). The majority of indigenous muskoxen in Greenland range within North and Northeast Greenland National Park and are protected from hunting except for emergencies. There is an annual hunting quota, allotted to local Inuit, of 200 animals from the population in Jameson Land, south of the Park. The rapid increase of the population translocated to Kangerlussuaq led to establishment of a hunting quota in 1988. The 1990 quota was 450 animals, although fewer animals were probably hunted (Boertmann et al., 1992).

Within Alaska, the five major populations of free-ranging muskoxen now total about 2300 animals. Harvest quotas have been established for three of these populations. Quotas on Nunivak and Nelson Islands are set in an attempt to maintain target populations of about 500 and 250 animals respectively (Smith, 1989). In 1991, 71 animals were harvested on Nunivak and 25 on Nelson Island (R. Kacyon, pers. comm.). Less than 10 animals are harvested annually from the ANWR population (P. Reynolds, pers. comm.). No hunting of muskoxen is allowed from the Seward Peninsula or Cape Thompson populations, although there may be some illegal hunting of the Cape Thompson animals (Smith, 1989).

Muskoxen are a conservation success story. The species which may have been close to extinction at the turn of the century now numbers over 100,000 individuals. Natural populations have recovered to secure numbers and translocated populations have become well-established on new ranges. However, the recent decline of

muskoxen in northeast Greenland indicates the species is still not immune from sudden fluctuations. The extreme climate of regions inhabited by muskoxen may predispose them to such fluctuations. Therefore, despite the present high numbers of muskoxen, care must be taken to closely monitor population trends and manage populations in accordance with localized hunting, predation and climatic pressures to prevent drastic population declines.

TAKIN (*BUDORCAS TAXICOLOR*)

The takin is a gregarious species living in mountainous regions of China, Bhutan, Burma and India. The genus and species was first described by Hodgson (1850), based on skins and skulls collected in the Mishmi Hills region of Assam, India. Hodgson supposed the takin to be related to the "gnoo" (*Connochaetes taurinus*), but also recognized similarities to the muskox (*Ovibos moschatus*). The takin and muskox are the only two extant species classified in the tribe Ovibovini within the subfamily Caprinae (Simpson, 1945).

Takin are large-bodied with a hump at the shoulders and sloping rump. The legs are stout and sturdy with pronounced dew claws above broad black hooves. The ears are small (125 mm) and protrude to the side. The short (< 100 mm), triangular tail has no tuft of hair on the end (Hodgson, 1850; Thomas, 1911b). The shaggy hair coat which varies from about 25 to 200 mm in length ranges from dark brown to golden yellow in color with considerable individual and subspecific variation. A short underwool is present, at least, during some of the year. The head of the takin is thickened dorsoventrally creating a "Roman-nosed" profile. Both males and females possess black horns that originate from a crest between the orbits and occipital plane (Neas and Hoffmann, 1987). In young animals, horns are straight, growing vertically at a slight outward angle. Mature animals have distinctive, transversely-ringed horns that are vertical at the base, turn outwards and then curve up and back to a point. Horns of females are slightly smaller than those of males.

Because of the paucity of published information on takin body morphology, size information will be presented for the species as a whole. Measurements of wild males include the following ranges: weights, 200 to 400 kg; height at shoulder, 975 to 1,500 mm; body length, 1,725 to 2,370 mm; length of horn, 350 to 630 mm; circumference of horn base, 319 to 375 mm and distance between horn tips, 200 to 319 mm. Measurements of wild females include: weights, 150 to 275 kg; height at shoulder, 900

to 1,200 mm; body length, 1,600 to 2,150 mm; length of horn, 356 to 406 mm, circumference of horn base, 225 to 250 mm and distance between horn tips, 181 to 219 mm (Hodgson, 1850; Hume, 1887; Thomas, 1911b; Bailey, 1912; Cooper, 1923; Sowerby, 1928; Smith, 1939; Wu, 1985).

The takin has a fundamental chromosome number (FN) of 60 (Bogart and Benirschke, 1975), as does the muskox (Tietz and Teal, 1967). The modal diploid number (2n) of the takin is 52 and the karyotype is 4 pairs of submetacentric and 21 pairs of acrocentric autosomes. The X is a large acrocentric chromosome and the Y is a small metacentric or submetacentric element (Bogart and Benirschke, 1975).

Most takins are social, living in groups that can exceed 100 (Bailey, 1912; Schaller et al., 1986), although most are smaller than 40 animals (Ge et al., 1990). These groups maintain their integrity despite the dense vegetation of the rugged mountains they frequently inhabit. Groups seasonally utilize more open alpine and sub-alpine habitats as well (Lydekker, 1908a; Wu et al., 1986). The pronounced dew claws that are well worn may contribute to the agility of the takin in covering steep, rocky mountain slopes and assist in moving through snow.

The remoteness and ruggedness of takin habitat combined with political factors have impeded collection of data on takins. Recent studies have begun to evaluate takin biology and status in China (Schaller et al., 1986; Wu et al., 1986; Ge et al., 1990; Wu, 1990; Groves, in prep).

Subspecies

Four subspecies of takins have been recognized. Differences between them have been based on physical characteristics and geographical separation of their ranges. The author is currently sequencing mitochondrial DNA from all subspecies in an attempt to define differences between them.

Golden Takin

Budorcas taxicolor bedfordi Thomas, 1911a: 27. Type locality Taibei Shan, Qinling Mountains, Shaanxi Province, China.

Initially described as a distinct species (Thomas, 1911a), this subspecies was discovered by Malcolm Anderson in 1910 while on an expedition sponsored by the Duke of Bedford. While there is considerable variation in coloration among individuals,

mature animals tend to be creamy white to golden yellow in color with black hair only at the tip of the muzzle. Old males may deepen to a reddish yellow. Calves are born dark, but the hair lightens to a grey tinged with yellow and a prominent dark dorsal stripe by 6 months of age.

The golden takin is limited to areas within the Qinling Mountains which extend from east to west for 300 km across southern Shaanxi Province, China (Sowerby, 1928; Wu, 1990). Within these mountains, the takins move seasonally between the river bottoms at 1,500 m to the mountain tops that extend up to 3,767 m (Wu, 1985).

At least three natural reserves provide some protection for golden takins and 1,300 to 2,000 animals may survive (Schaller, 1985; J. Wu , pers. comm.). However, their distribution is patchy and habitat destruction, including lumbering, bamboo harvesting and road building, limits the ability of animals to find adequate food resources and move into new environments. Despite the protected status of the takin within China, poaching, both with snares and guns, is not uncommon (pers. obs.) and may be having an impact on population numbers.

Mishmi Takin

Budorcas taxicolor taxicolor Hodgson, 1850:65. Type locality Mishmi Hills, Assam, India.

Specimens from this subspecies in the eastern Himalayas provided the first descriptions of the genus and species to the western world. The body of these animals tends toward yellowish grey. Extremities, including head, neck, tail and legs are black. A dark, dorsal stripe may be apparent and ventral surfaces are usually darker than dorsal surfaces (Lydekker, 1908b; Bailey, 1912).

The range of the Mishmi takins includes the Mishmi Hills of northern Assam, India (Hodgson, 1850) and extends north into southeast Tibet, China east of the Yarlung Zangbo (Brahmaputra) River (Bailey, 1912; Bryant, 1923; Wu, 1985). The range extends into Burma north of Putao and west of the Irrawaddy-Salween divide (Cuffe, 1914; Hla Aung, 1967). This subspecies also occurs in a narrow strip of Yunnan Province, China west of the Nujing River (Wu, 1985). Within their range, Mishmi takins utilize areas from 1,000 m up to at least 4,500 m (Cuffe, 1914; Cooper, 1923).

No estimates of populations for this subspecies have been published. In 1967, the Mishmi takin was described as "not rare" within Burma (Hla Aung, 1967), but the lack of reports of recent sightings indicates numbers are probably low. Within the Chinese range of the Mishmi takin, less than 1,000 are thought to survive (J. Wu, pers. comm.).

Sichuan Takin

Budorcas taxicolor tibetana Milne-Edwards, 1868:367 (not seen, cited in Neas and Hoffmann, 1987). Type locality Moupin (*sic*), Sichuan Province, China.

The Sichuan takin has coloring intermediate between the Mishmi and golden takins. Both seasonal changes and sexual dimorphism in coloration contribute to color variations within this subspecies (Lydekker, 1908b). Forequarters of mature animals range from reddish-brown to yellow with the hindquarters darker and grayer. The limbs also may be predominantly dark. A dark dorsal stripe often extends from the rump up along the back. Ears are usually dark and black hair extends from the nostrils up the muzzle towards the eyes. Females tend to be paler and grayer than males (Lydekker, 1908a). The horns of Sichuan takins may be more slender than those of Mishmi takins (Lydekker, 1908b).

Despite the subspecific name which was based on mistaken geographic information (Lydekker, 1908b), the range of *B. t. tibetana* is largely within western Sichuan Province, China and does not appear to extend west into Tibet. The northern extent of the range is within southern Gansu Province, south of the Bailong River in the Min Mountains. Sichuan takins extend south through Sichuan Province in the Qionglai, Daxue and smaller mountain ranges into a small portion of northern Yunnan Province north of the Yangtze River (Young, 1948; Schaller, 1985; Wu, 1985). Within their range, Sichuan takins utilize areas ranging between 1,500 and 4,000 m (Wu, 1985; Schaller et al., 1986).

With a more extensive range, the Sichuan takin is probably the most numerous subspecies. Schaller (1985) estimates several thousand survive in the wild, with the highest densities being in parts of the Min and Qionglai Mountains. These animals benefit from sharing habitat with the giant panda (*Ailuropoda melanoleuca*). At least 12

reserves have been created to protect the pandas and these incidentally protect takins as well. Takin readily utilize secondary growth in the reserves where lumbering is no longer allowed and, in such areas, numbers may be increasing (Schaller et al., 1986). However, despite the protected status of the takin, there is poaching of these animals in Sichuan (D. Reid, pers. comm.).

Bhutan Takin

Budorcas taxicolor whitei Lydekker, 1907:887. Type locality Chumbi River, Bhutan.

This subspecies tends toward brown coloration with the underparts and limbs almost black. A black dorsal stripe extends the length of the back to the head. Most of the head is black as well. Few descriptions of this subspecies have been published, but the Bhutan takin appears to have darker coloring than the Mishmi takin (Lydekker, 1908b; Wu, 1985). According to Pocock (1913), Bhutan and Mishmi takins have similar coloring and individual and seasonal variation account for observed differences between the subspecies. Lydekker (1908b) reported the Bhutan takin to have a smaller body and horns than the Mishmi takin.

Historically, this subspecies was found as far west as Sikkim, India and western Bhutan (Lydekker, 1906, 1907) with the eastern boundary of the range defined by deep river gorges (i.e. tributaries of the Brahmaputra) west of the Mishmi Hills which maintained a geologic barrier between the Bhutan and Mishmi takins. Within Tibet, Bhutan takins range southwest of the Yarlung Zangbo River at least as far west as Comai County (Wu, 1985). The range may extend south into the Miri Hills in Assam, India east of Bhutan. Bhutan takins in Tibet have been found at elevations between 3,500 and 4,500 m (Wu, 1985). At the southern extremes of their range on the edge of the Himalayan plateau, they may utilize lower elevations.

Extremely little is known about the population status of the Bhutan takin, but numbers are probably low. Within Tibet, where the takin is officially protected, 500 animals may survive (J. Wu, pers. comm.).

Synonyms

Budorcas was derived from a combination of the Greek words, "bous" and "dorkas," meaning cow and gazelle. *Taxicolor* stems from the Latin "taxus" for badger and refers to the badger-like color of the animals first described by Hodgson (1850).

The name takin was adopted from the name for the animal used by the local people in the Digaru region of the Mishmi Hills where the species was first reported (Hodgson, 1850). Other names for the species used by Mishmi peoples include "kin", "kyem", "akron", and "sibeno" (Hodgson, 1850; Bailey, 1912). The official Chinese name for the takin is "lingniu" or antelope cow. Other local Chinese names include: "baiyang" or white sheep; "yeniu" or wild cow; "zhuniu" or bamboo cow and "panyang" or curved horn sheep (Wu, 1990). Within Tibet, the takin may be referred to as "ya go", "shing na" or "jie men ya" (Bailey, 1912; Wu, 1990).

Status

The remoteness, ruggedness and dense vegetation of most takin habitat has made assessing population status difficult. Because of political constraints, the difficult terrain takins inhabit and associated logistical expenses, most areas of takin habitat have not been accessed by biologists interested in surveying populations. Without evidence to the contrary, takin numbers must be considered low.

Within China, the takin has been granted "First Class Protected Animal" status along with the giant panda and golden monkey (*Rhinopithecus roxellanae*). Reserves have been established for takins both in Sichuan and Shaanxi Provinces. Additional reserves for giant pandas and golden monkeys offer protection to some takins as well since the ranges of these species overlap. Within some of the reserves, particularly the panda reserves in Sichuan Province, takin numbers may be increasing (Schaller et al., 1986; Ge et al., 1990). However, poaching, particularly snaring, is common even within reserves. Snarers who hope to kill musk deer (*Moschus sifanicus*), the glands of which can be sold for significant profit, also kill takins and other non-target species. Local people do shoot takins for meat. Unfortunately, in China little money is available to police the reserves and enforce anti-poaching laws.

In addition to poaching, habitat destruction is having an impact on takin populations. Takins utilize mountain slopes heavily-vegetated with mixed deciduous

and coniferous forests and thick stands of bamboo. Lumbering is an important industry for impoverished mountain villages in China. Bamboo is harvested by clear-cutting entire slopes. Expanding human populations in the mountains have resulted in more land being cleared for agriculture and new roads being pushed through the mountains. Habitat destruction leads to fragmentation of takin habitat which may result in isolated pockets of small populations that are not viable.

The IUCN has classified the status of the Sichuan takin as indeterminate and the golden takin as rare. The Bhutan and Mishmi takins have no official status, but are probably subject to pressures of poaching and habitat destruction. The extreme remoteness of their ranges may offer some protection to these subspecies. Systematic surveys should be conducted to properly define the status of all subspecies.

CHAPTER 2 A SOCIAL UNGULATE IN DENSE HABITAT: GROUP SIZE AND HABITAT USE OF THE GOLDEN TAKIN

INTRODUCTION

The takin (*Budorcas taxicolor*) is a large, social ungulate that lives in mountainous regions of China, Burma, Bhutan and India (Schaller, 1977). The species was first described by Hodgson (1850) who supposed it to be related to the "gnoo" (*Connochaetes taurinus*), but also recognized similarities to the muskox (*Ovibos moschatus*). The takin is classified within the family Bovidae and subfamily Caprinae (Simpson, 1945), but recent DNA studies have placed it closer to the sheep and goats than to its previously accepted position in the tribe Ovibovini with the muskox (Groves and Shields, 1995).

The recognized subspecies of takin, golden (*B. t. bedfordi*), Sichuan (*B. t. tibetana*), Mishmi (*B. t. taxicolor*) and Bhutan (*B. t. whitei*), all have geographically distinct ranges (Fig. 2 - 1; Neas and Hoffmann, 1987). All subspecies occupy mountainous habitats characterized by their remoteness, ruggedness and dense vegetation below treeline. European adventurers seeking to collect takin specimens in the early 1900's describe extreme travails in traveling to takin habitats, the challenges of moving through that habitat and high levels of frustration in attempting to see and shoot takins (Bailey, 1912; Anderson, 1920; Andrews, 1922a; Kaulbach, 1935; Whittall, 1935).

These logistical difficulties as well as political restrictions have limited attempts to investigate the ecology of the takin. Within China, the Sichuan and golden takins have the same protected status as, and share habitat with, the giant panda (*Ailuropoda melanoleuca*). Partially because of this association, some recent efforts have been made to study takin ecology (Schaller et al., 1986; Wu et al., 1986; Ge et al., 1990), but knowledge of the social behavior and habitat use of this species is limited.

The takin appears to be an unusual ungulate in that it is large (200 - 300 kg; Wu, 1985) and gregarious, yet lives in dense habitat (Bailey, 1912; Schaller, 1977). Among most ungulates, group size tends to decrease with increasing habitat density

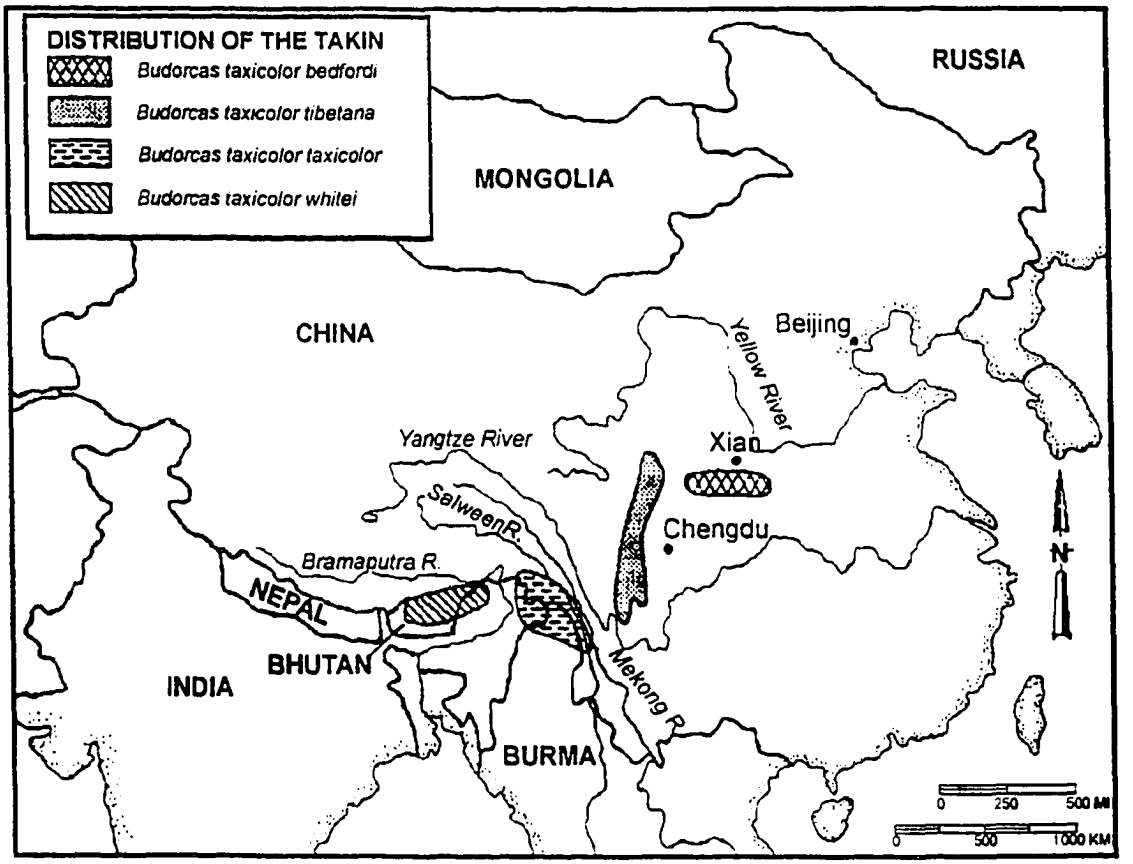

Figure 2 - 1. Distribution of the four subspecies of takins. This study focused on the golden takin (*B. t. bedfordi*), south of the city of Xian.

(Alexander, 1974) and larger animals tend to live in more open habitats (Leuthold, 1977). Feeding styles are not directly correlated with body size (Hofmann, 1989), but large body size can contribute to the ability to digest lower-quality foods so large ruminants may be more general in dietary selection than smaller ones (Hudson, 1985). Gregarious ungulates also tend to be less selective in feeding preferences than solitary ones (Jarman, 1974).

To better understand aspects of the ecology of takins, I undertook a field study of a population of golden takins in Shaanxi, China. The objectives of this study were to investigate group size in relation to habitat, define patterns of habitat use and dietary selection within the area. I hypothesized group size would vary with vegetation density and that takins would selectively utilize less dense areas within the habitat, but consume a wide variety of plant species.

STUDY AREA

The study area in Ningshaan County within the Qinling Mountains in Shaanxi Province, 150 km south of the city of Xian at 33^{o} N latitude and included 35 km^2. The boundaries of the area were defined by mountain ridges, streams and a road on the eastern edge. Elevations within the area range from 1,600 to 2,530 m. Terrain is steep and rugged with ten major stream valleys. Virtually all slopes are densely vegetated with mixed broadleaf and evergreen forests. Thick stands of bamboo cover some higher slopes. Alpine meadows occur in scattered locations near the highest elevations, but most of the area is below treeline. Small meadow areas near some streams appear to be the result of human activity before 1950.

Human Activity

There are no permanent human residences in the study area. However, some of the area is used by humans during summer. From June to October, local villagers tap *Rhus verniciflua* trees for sap that is made into lacquer. These men live in simple camps and make small trails through the area. Other villagers visit the area to collect plants used to make traditional medicines. They use existing trails and seldom spend more than two days at a time in the mountains. Although illegal, snaring of wildlife is a common activity in the area. The men who set snares often establish small camps, but use existing game trails. During 1989, an area of about 0.5 km^2 was clear cut for

bamboo. There was no active lumbering in the area during the period of study, but a large lumbering operation was located just south of the area and the workers were known to hunt within the area.

Other Mammal Species

Live mammals seen in the area included masked palm civet (*Paguma larvata*), yellow-throated marten (*Martes flavigula*), Père David's rock squirrel (*Sciurotamias davidianus*), bamboo rat (*Rhizomys sinensis*) and Asiatic chipmunk (*Eutiamias sibiricus*). Signs of other smaller ungulates: musk deer (*Moschus sifanicus*), goral (*Nemorhaedus goral*) and muntjac (*Muntiacus reevesi*) were seen frequently and dead specimens were found in snares. Signs of large carnivores were not common, but tracks and feces of Asiatic black bear (*Ursus thibetanus*), Asiatic wild dog (*Cuon alpinus*), leopard (*Panthera pardus*) and leopard cat (*Felis bengalensis*) were seen. The last tiger (*Panthera tigris*) believed to be in the area was killed in 1964 (J. Wu, pers. comm.). Giant pandas were present until the early-1980's when a bamboo die-off forced them to move from the area. The bamboo has recovered, but pandas have not returned.

Weather

During the periods of the study from late July through late November, temperatures in the study area ranged from highs of 30° C to lows of -10° C. Precipitation fell on 40% of the days, including snow that did not persist in early October, early November and late November. Low clouds and fog, which limited visibility, were common in October and November.

METHODS

Field studies were conducted July - November, 1988 and October - November 1990. Base camps were established at elevations of 1,720 m in 1988 and 2,380 m in 1990. Daily surveys (weather permitting) were conducted within the study area to search for takins and signs of takin activity.

Field procedures included following recent signs of takin activity that were encountered during systematic surveys through the study area. If takins were sighted (direct observation), the number of animals in the group was counted. Habitat

variables, including elevation and vegetation density were recorded. Vegetation density was defined by four classes: 1 = < 1 stem/m^2, 2 = 1 - 5 stems/m^2, 3 = 6 - 10 stems/m^2, 4 = > 10 stems/m^2. When takins were not sighted, as was common, searches were made to locate an area where the group had bedded. These areas could be distinguished by compressed vegetation. Estimates were made of the group size by counting the number of beds (indirect observation). The same habitat variables were recorded at these bedding sites as for direct observations. In analyzing group size in relation to habitat, each group observation, direct and indirect, was treated as an independent event. Differences between group sizes in the four density categories were tested by a one-way analysis of variance.

In 1988, a survey of habitat use was conducted by using transects 65-m long, 100 m apart and perpendicular to ridgelines or stream valleys. Along each transect, five plots, each 5 by 5 m and 10 m apart were sampled. A total of 447 plots were sampled. For each plot, data on 15 variables were recorded as well as evidence of takin feeding, feces, or beds (Table 2 - 1). Step-wise logistic regression (Dixon, 1985) was used to develop a model with the variables most critical in distinguishing between plots used and not used by takins. Multicolliniarity between variables was controlled for by eliminating one of any pair of variables with $r \geq |0.50|$. Multivariate analysis of variance was used to test the significance of variables that entered in the logistic regression model. The significance of variables indicates takins select for or against use of those parameters within their habitat (Bowyer et al., 1995).

In 1990, 90 random transects, each 30-m long, were sampled to evaluate takin use of feeding habitat. In addition to the 11 habitat variables that were recorded (Table 2 - 2), presence or absence of signs of takin feeding were noted for each linear transect. Analysis of transect data to distinguish between habitat with and without takin feeding was conducted in the same fashion as for the 1988 data.

Plant species observed to have been eaten by takins were identified during both years of study. During 1990, plant species at 1-m intervals along each transect also

Table 2 - 1. Summary of habitat use variables of 447 plots characterized as used or not used by takins ($\overline{X} \pm 1$ SD) measured during 1988 survey in Qinling Mountains, China.

Variable	Plots used by takins (n=222)	Plots not used by takins (n=225)
Elevation (m)	2203.4 ± 160.7	2113.8 ± 175.7
Density [a]	2.09 ± 0.89	2.36 ± 0.93
# of trees	3.61 ± 2.68	3.13 ± 2.35
# of fallen trees	0.71 ± 1.05	0.91 ± 1.11
Patchiness [b]	0.51 ± 0.50	0.48 ± 0.50
Valley [c]	0.42 ± 0.50	0.64 ± 0.48
Trees [c,d]	0.90 ± 0.31	0.86 ± 0.35
Shrubs [c,d]	0.96 ± 0.20	0.95 ± 0.23
Forbs [c]	0.94 ± 0.24	0.89 ± 0.31
Grasses [c]	0.74 ± 0.44	0.69 ± 0.46
Bamboo [c]	0.14 ± 0.36	0.21 ± 0.41
Rocks [c]	0.18 ± 0.38	0.18 ± 0.38
Water [c]	0.05 ± 0.22	0.08 ± 0.26
Human use [c]	0.05 ± 0.23	0.12 ± 0.33
Trail	0.44 ± 0.50	0.32 ± 0.47

a Density coded as 1 = <1 stem/m^2, 2 = 1 - 5 stems/m^2, 3 = 6 - 10 stems/m^2 and 4 = > 10 stems/m^2.

b Coded as 1 = patchy plant distribution and 0 = even plant distribution.

c Coded as 1 = present and 0 = not present.

d Trees defined as having woody stems > 10 cm in diameter and/or > 3 m high; shrubs defined as having woody stems < 10 cm in diameter and/or < 3 m high.

Table 2 - 2. Summary of variables characterized by sign of feeding or no feeding by takins ($\bar{X} \pm 1$ SD) of 90 transects measured in Qinling Mountains, China in 1990.

Variable	Feeding (n = 58)	No Feeding (n = 32)
Elevation(m)	2161.2 ± 243.1	2148.3 ± 242.7
Density [a]	2.49 ± 0.89	2.20 ± 0.90
Relief [b]	1.91 ± 0.86	1.69 ± 0.90
Water [c]	123.2 ± 86.4	121.4 ± 87.5
Trees [d,e]	0.88 ± 0.33	0.81 ± .40
Shrubs [d,e]	0.76 ± 0.43	0.94 ± 0.25
Forbs [d]	0.97 ± 0.18	0.75 ± 0.44
Grasses [d]	0.95 ± 0.22	1.00 ± 0
Bamboo [d]	0.45 ± 0.50	0.34 ± 0.48
Rocks [d]	0.02 ± 0.13	0 ± 0
Human Use [d]	0.45 ± 0.50	0.38 ± 0.49

a Coded as 1 = <1 stem/m^2, 2 = 1 - 5 stems/m^2, 3 = 6 - 10 stems/m^2 and 4 = > 10 stems/m^2.
b Coded as 1 = valley, 2 = slope and 3 = ridge.
c Distance (m) to closest water.
d Coded as 1 = present and 0 = not present.
e Trees defined as woody stems > 10 cm diameter and/or > 3 m high; shrubs < 10 cm diameter and/or < 3 m high.

were identified. David Murray of the University of Alaska Museum, Fairbanks AK, David Boufford of the Harvard University Herbaria, Cambridge MA, Ming-Jun Sun at the Holden Arboretum, Mentor OH and the staff at the Shaanxi Institute of Zoology helped with plant identification and translation of Chinese plant names into Latin.

Statistical analyses were conducted using software produced by BMDP Statistical Software, Inc. (Dixon, 1985).

RESULTS

Group Size

Opportunity for direct observation of takins was limited by their high mobility and the dense vegetation. Groups of takins, however, left obvious trails through the vegetation that were easy to follow. These trails could be dated by condition of feces and the degree of wilting of disturbed vegetation. Even when following a fresh trail, sighting the group was unlikely. Over the course of the study, direct observations of

Table 2 - 3. Takin group size observations in Qinling Mountains, China.

Year	Type of observation	Number of observations	Range of group size	Mean group size ± 1 SD
1988	Direct	10	1 - 40	11.3 ± 12.3
1988	Indirect	23	1 - 37	7.1 ± 7.5
1990	Direct	14	1 - 5	2.1 ± 1.4
Both	Both	47	1 - 40	6.5 ± 8.2

takins were made 24 times and indirect observations 23 times. Group sizes ranged between 1 and 40 individuals (Table 2 - 3).

An analysis of variance of mean group size in relation to vegetation density showed no significant difference between group sizes in the different habitats (F = 1.86, P = 0.15), although there was a tendency for groups to be larger in less dense habitat (Fig. 2 - 2). There was no significant relationship between elevation and group size, but there was a trend for larger groups to be at higher elevations (Fig. 2 - 3).

Habitat Use - 1988

Of the 447 plots sampled, 222 showed signs of use by takins. Of the variables measured in this survey, elevation and presence of a valley were negatively correlated (r = -0.60) and the number of trees and presence of trees were positively correlated (r = 0.50), so the valley and number of tree variables were eliminated before the step-wise development of the logistic model. This logistic model, which correctly classified 67 % of the plots, included elevation, vegetation density, trail, forbs, and trees as variables. The model took the form:

$$\log(\pi(x)/1-\pi(x)) = 8.186 - 0.00347\text{Elevation} + 0.347\text{Density} - 0.494\text{Trail} - 0.615\text{Trees} - 0.808\text{Forbs}$$

where π is the probability a plot was used by takins.

Multivariate analysis of variance of these variables indicated elevation, density, and trails were significantly different between used and unused plots (Fig. 2 - 4). These data suggest takins tend to use higher elevations and less densely vegetated areas, and that they may utilize existing trails.

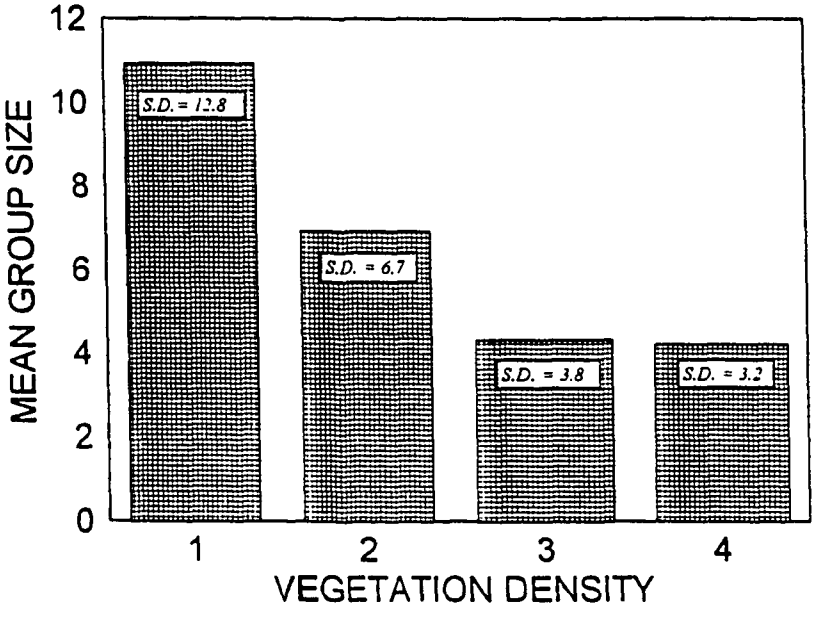

Figure 2 - 2. Mean takin group size in relation to vegetation density. Standard deviations are in boxes on bars. Density classes: 1 = < 1 stem/m^2, 2 = 1 - 5 stems/m^2, 3 = 6 - 10 stems/m^2, 4 = > 10 stems/m^2. There was no significant difference between group size in the different density classes.

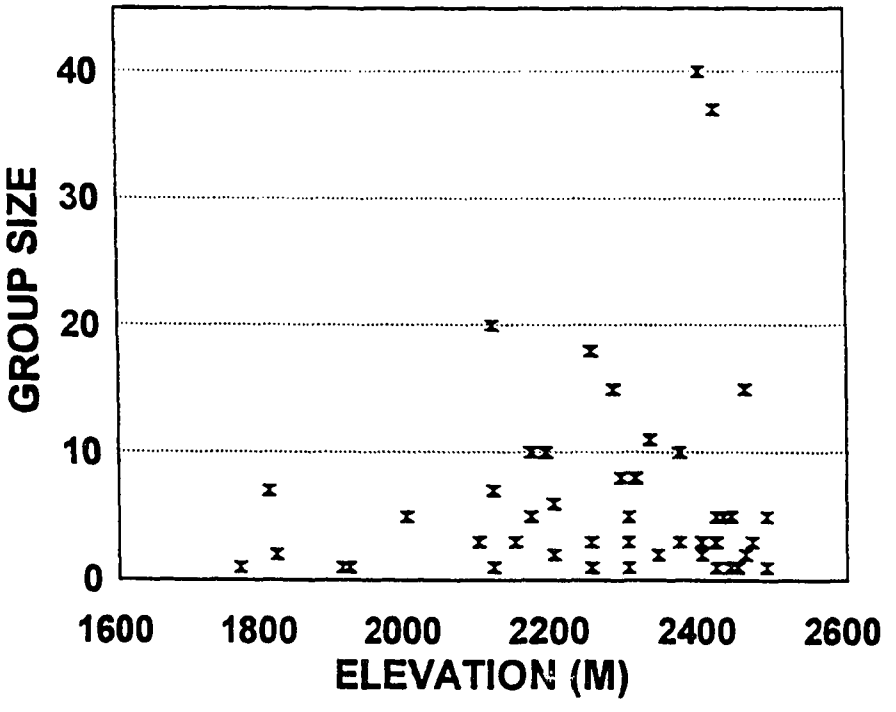

Figure 2 - 3. Takin group size in relation to elevation. There was no significant relationship between group size and elevation.

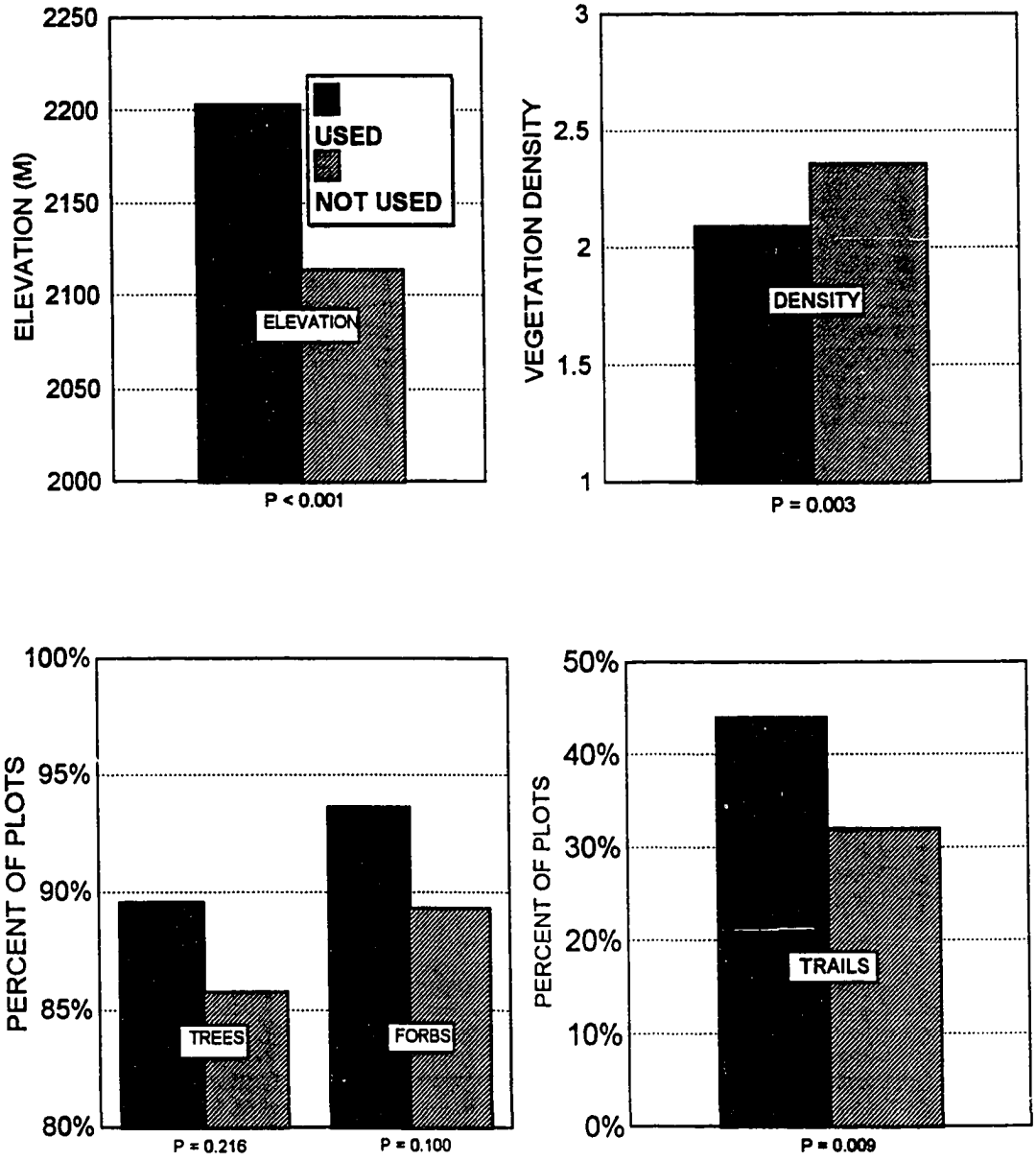

Figure 2 - 4. Variables which entered into stepwise logistic regression model classifying plots used (N = 222) and not used (N = 225) by takins. *P*-values are from multivariate analysis of variance using these variables.

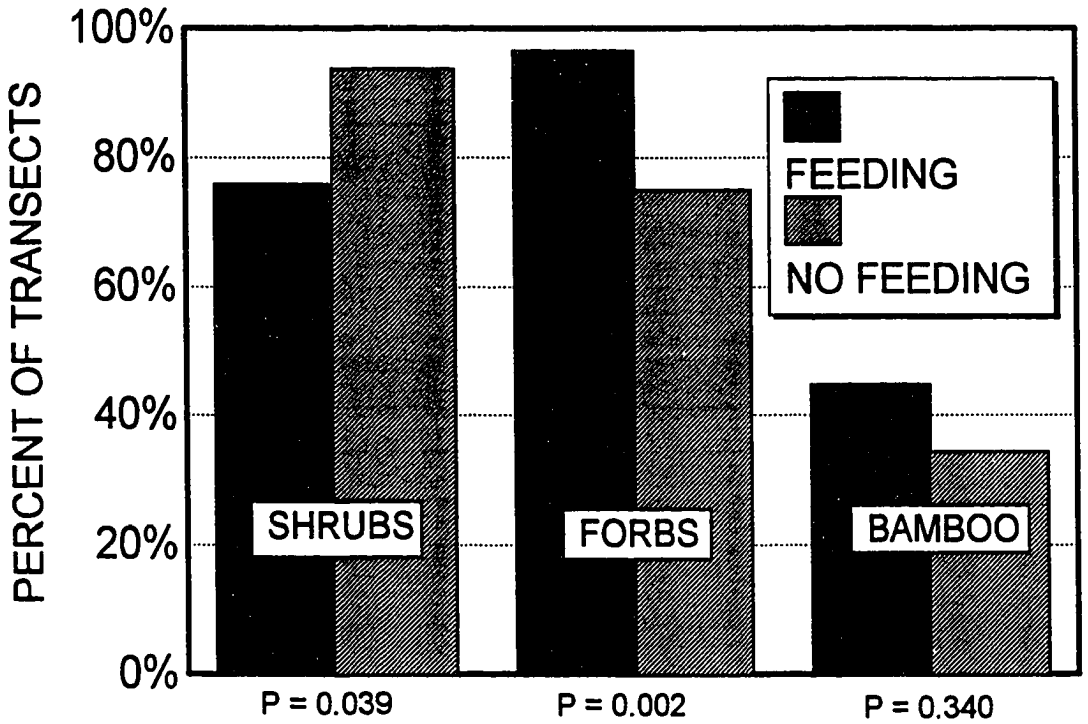

Figure 2 - 5. Variables which entered into stepwise logistic regression model classifying transects with signs of takin feeding (N = 58) and with no signs of feeding (N = 32). *P*-values are from multivariate analysis of variance using these variables.

Feeding Use Survey - 1990

Feeding signs of takins were observed along 58 of the 90 transects. Of the variables measured, elevation was positively correlated with relief ($r = 0.53$) and distance to water ($r = 0.70$) and negatively correlated with human use ($r = -0.57$) and, consequently, the elevation variable was removed before the logistic model was developed. This model which included shrubs, forbs and bamboo as variables, correctly classified 68 % of the transects and took the form:

$\log(\pi(x)/1-\pi(x)) = 0.615 + 1.758\text{Shrubs} - 2.510\text{Forbs} - 1.269\text{Bamboo}$, where π is the probability a transect had takin feeding sign.

Multivariate analysis of variance of these variables indicated that only forbs and shrubs were significantly different between transects with feeding and those without signs of feeding (Fig. 2 - 5). Transects with feeding tended to have fewer shrubs and more forbs than those without feeding.

Plant Species

During the 1990 survey, 120 plant species from 88 genera and 44 families were identified (Table 2 - 4). Of these species, only 15 showed no evidence of being eaten by takins. Takins consumed a variety of plant parts, including flowers, leaves, current annual growth, seeds, bark, twigs and small branches. Shrub stems up to eight cm in diameter were frequently broken over by takins to enable browsing of upper branches.

DISCUSSION

Group size

Tendencies for larger groups to be observed in less dense habitats and at higher elevations may be related to better visibility in those locations. Nonetheless, indirect observations, which were not influenced by visibility, also suggest larger groups tend to be at higher elevations and in less dense habitats. Large groups (> 15 animals) were observed to travel through extremely dense vegetation, and examination of their trails indicated they fed on the dense vegetation as they traveled, if they were undisturbed. This suggests factors in addition to elevation and habitat density are involved in the control of group size.

Takins were wary of humans and if startled, or approached too closely, would rapidly disappear from sight into the dense vegetation. Most Europeans attempting to

hunt takins have commented upon the speed with which takins travel through the dense forests and their agility in moving over the rugged terrain (Andrews, 1922b; Sowerby, 1928; Whittall, 1935). Several times takins could be heard and fresh tracks and feces were observed, but the animals were never seen. Because of difficulties in observing takins, data on group size were obtained opportunistically rather than systematically, and cannot be expected to define statistically significant relationships between group size and habitat.

Most reports of takins suggest they are gregarious except for older males that may be solitary (Whittall, 1935; Schaller et al., 1986; Ge et al., 1990). Groups as large as 300 have been described (Bailey, 1912), although groups of 5 to 40 appear to be more common (Cooper, 1923; Schaller et al., 1986; Ge et al., 1990). Formation of groups of $\geq$ 40 animals may be seasonal and temporary. Bailey (1912) reported that Mishmi takins formed a group of 300 animals in summer and split into groups of 10 to 20 in winter. In contrast, Sichuan takins reportedly form larger herds in winter and spring and smaller groups in summer (Schaller et al., 1986; Ge et al., 1990). This study took place during July to November, so no data are available on group sizes throughout the rest of the year. My observations, however, suggest that larger groups were temporary aggregations. A group of $\geq$ 40 takins leaves an obvious trail through the vegetation and feeding sites of these groups are well-trampled with many broken shrubs. Signs of such large groups were not frequently encountered, whereas signs of smaller groups (about 5 - 15) were regularly observed. Prior to the sighting of a group of 40 takins, I observed tracks of several smaller groups all heading toward the area where the large group was seen. This group was disturbed while being observed and divided into two groups which moved off in separate directions.

Although groups of 1 - 40 takins were observed in 1988, groups of only one to five were seen in 1990. The 1990 field season overlapped with the time in October 1988 when large groups were seen, so this difference does not appear to be merely seasonal. Fewer signs of recent takin activity were observed throughout the study area in 1990 than 1988. I believe the density of takins within the study area decreased over this period. Some of the decrease may be attributed to natural mortality, predation, hunting and snaring, but because such mortalities did not appear to be frequent, I

propose some takins moved away from the area. This movement may have been precipitated by increased human activity, particularly the harvesting of bamboo within the area.

Habitat use

Results of the 1988 and 1990 surveys suggest elevation, vegetation density, and the presence of existing trails influenced habitat use by takins, whereas the relative presence of forbs and shrubs were the most important variables for classifying areas where takins fed. Both logistic models classified > 60 % of the samples correctly, but neither is strong enough to provide a clear understanding of how takins use their environment. These results suggest that within the study area, takin use of available habitat is not strongly influenced by any of the variables measured.

As with group size, there may be seasonal changes in habitat use and feeding site selection that were not detected because of the time frame of this study. Some signs of feeding, particularly grazing or browsing on new spring growth, are not easily detected after the passage of time. Likewise, the loose, soft feces takins produce in summer are washed away after one or two rainstorms. In contrast, the browsing of large shrubs and breaking of branches or eating of bark might be evident from one year to the next. Pelleted takin feces of winter can last more than one year, although sometimes they may be buried under leaf litter. Thus, the surveys conducted may not have detected spring and early summer activity of takins.

Takins have been reported to have seasonal movements, mostly along a vertical axis. Within their ranges, takins tend to frequent lower elevations in winter and higher elevations in summer (Sowerby, 1928; Schaller et al., 1986; Wu, 1990). The range of elevations varies with geographical location, the western subspecies on the edge of the Himalayan plateau may never descend below 2,500 m (Kaulbach, 1935), whereas the highest elevations attainable for the eastern subspecies are about 3,000 m (Wu, 1990).

During the course of this study, I observed takins or signs of recent takin activity in all but the lowest valleys of the study area. The animals seemed to use all of the forested slopes within the area. Plant species composition of the slopes varied with elevation, but density was quite homogeneous except for some scattered meadows.

These meadows were the only areas where obvious signs of takin feeding were not observed. In autumn, some takins were observed crossing alpine meadows and resting along the edges, but they always moved into the shrubs to feed. Local mountain people reported they sometimes saw takins in alpine meadows and in the few meadows on lower slopes in spring when calves were young. Signs of use of meadows in spring would not likely be visible by late summer.

Forage preferences

The list of plant species (Table 2 - 4) is not a complete list for the area due to the seasonality of the study and the diversity of species in the area. The 105 species observed to have been eaten by takins is, therefore, a minimum list for this area. Schaller et al. (1986) listed 138 species eaten by Sichuan takins and Wu et al. (1986) reported 114 species eaten by takins throughout their range. Grasses and grass-like plants comprise a small percentage (10%) of the total number of species reported eaten, indicating takins are primarily browsers. The length of the list confirms that takins are generalists consuming a wide variety of foods. That takins browse more than graze is supported by my observations that meadows are used little in contrast to the more densely-vegetated areas.

Results of this study of takins within the Qinling Mountains indicate that they are generalists that use the breadth of their habitat and consume many of the diverse plant species within that habitat. The large body size of takins is adaptive to this generalist lifestyle. Because of their large bodies, and presumably large rumen and digestive system, takins should be able to digest low quality foods such as coarse browse and bamboo (Hudson, 1985) in addition to more succulent forbs and grasses and thus adapt to seasonal changes in vegetation availability and quality. Takins readily break over large shrubs and small trees to reach the leaves on upper branches. This ability makes more forage available to them than would be available to smaller ungulates. Additionally, their large bodies are adapted to moving rapidly throughout the area and to navigating steep slopes and rock faces common in the area. Thus, takins have access to a vast variety of plant species over a wide area throughout the year.

Feeding styles of ungulates are often related to group size because of the effect of feeding style on food availability and dispersal (Jarman, 1974). In a "coarse-grained"

environment such a forest, in which the feeding of one animal reduces food availability by removing entire food items, Jarman (1974) predicted animals would be widely dispersed and not tend to form groups. Takins apparently contradict this prediction. Due to the feeding style of takins, which is general and flexible, and the lush forests at temperate latitudes that produce abundant forage, food availability does not appear to limit the ability of takins to form groups.

Environmental funneling due to topographic features, food and water distribution and reproductive behavior has been suggested as the cause of bison (*Bison bison*) forming apparently random, large groups (Lott and Minta, 1983). These factors do not seem to influence takin group formation, however. Takins are not constrained in their movements by topographic features. They readily traveled over the steepest slopes in the area and were observed rapidly climbing up nearly vertical rock faces > 3 m high. While there was a tendency for takins to use areas with existing trails (Fig. 2 - 4), this trend was not strong and the animals readily traveled off trails as well. Thus trail use cannot explain formation of takin groups. The largest groups were observed at higher elevations, but small groups also were commonly observed at these same elevations, so elevation does not funnel takins into large groups. Results of the habitat use and feeding use surveys indicate takins utilize the entire area. Running streams are distributed over the entire area. Thus, takins do not need to concentrate in limited areas where food and water is available and consequently be funneled into groups. Few observations have been made of the reproductive behavior of wild takins, but they reportedly form harems (Wu, 1990). These harems may contribute to formation of groups during the mating season in late summer (Neas and Hoffmann, 1987), but because groups are observed throughout the year, factors in addition to reproductive behavior must contribute to group formation in takins.

The gregarious behavior of these takins in their densely vegetated environment is still an enigma. A major advantage of gregarious behavior is increased predator detection and consequent decreased vulnerability to predation (Bertram, 1978). Prey animals in a group also may have a lower probability of being detected visually by a predator than the same number of animals scattered randomly in open habitats (Vine, 1971, 1973). This lower probability of detection may extend to dense habitats in which

predators might depend on scents and sounds to detect prey. Predator signs were observed along the same trails takins used. If these predators were randomly searching for prey, an extrapolation of the model of Vine (1971) would predict they would be less likely to encounter a group of prey animals than scattered individuals. For these advantages of gregarious behavior to be realized, however, the group must be able to function as a unit (Jarman and Jarman, 1979). Dense vegetation which limits visibility can hinder detection of predators and visual communication between group members and thus may limit the advantages of gregarious behavior. The ability of takins to move rapidly and quietly through thick shrubs or bamboo as a cohesive unit is remarkable. When takins were moving through dense vegetation, they could be heard making vocalizations which may aid in maintaining group cohesion. The smaller ungulates in the area live singly or in pairs and are cryptically colored (Schaller, 1977). These species may avoid detection by predators by being inconspicuous and employing a hiding strategy (Jarman, 1974; Hirth, 1977). Takins are too big to hide from predators, but can depend on the group to avoid predation. Despite the dense vegetation, groups may help to detect threats in time to evade them and group members can help defend each other from predators (Jarman and Jarman, 1979).

Certainly takins were wary and individuals in a group would snort in alarm when humans were detected. The animals would then run together and face the intruders. Takins will form a tight group and face as a unit predators such as wild dogs (Wu, 1990). The local people report being charged by takins and are unwilling to approach them closely unless armed. Among ungulates, group defense is only observed in species such as the African buffalo (*Syncerus caffer*) muskox and takin (Schaller, 1977; Sinclair, 1977; Gray, 1987), which are large enough to repulse predators and have horns that can be used as effective weapons against predators. I propose the adaptive advantage of group living to avoid predation, in particular group defense, appears to be a significant force in selecting for gregariousness in takins despite their dense habitat and large body size. Due to the abundant forage distributed throughout the area and the generalist feeding style of takins, their gregarious behavior is not limited by food availability.

Table 2 - 4. Plant Species identified at 1-m intervals along 90 random transects in Qinling Mountains, China in 1990.

FAMILY	GENUS	SPECIES	EATEN[a]
Aceraceae	*Acer*	*caudatum*	
	Acer	*oliverianum*	
	Acer	*truncatum*	
Actinidiaceae	*Actinidia*	*chinensis*	
	Actinidia	*kolomikta*	
Alliliaceae	*Allium*	*ovalifolium*	
Anacardiaceae	*Cotinus*	*coggygria*	
	Rhus	*chinensis*	
	Rhus	*verniciflua*	
Araliaceae	*Acanthopanax*	*giraldii*	
	Acanthopanax	*leucorrhizus*	
	Aralia	*chinensis*	
Aspidiaceae	*Cyrtomium*	*fortunei*	
	Matteuccia	*sp.*	
Berberidaceae	*Berberis*	*circumserrata*	
	Berberis	*dolichobotrys*	
	Berberis	*shensiana*	
	Epimedium	*grandiflorum*	
Betulaceae	*Betula*	*albo-sinensis*	
	Betula	*septentrionalis*	
Caprifoliaceae	*Lonicera*	*microphylla*	
Celastraceae	*Euonymus*	*alatus*	
	Euonymus	*microcarpus*	
	Euonymus	*phellomanus*	
Compositae	*Cacalia*	*hastata*	
	Cacalia	*sp.*	
	Synurus	*deltoides*	

FAMILY	GENUS	SPECIES	EATEN[a]
Cornaceae	*Cornus*	*hemsleyi*	*N*
Cruciferae	*Cardamine*	*leucantha*	
	Cardamine	*tangutorum*	
Cupressaceae	*Juniperus*	*taiwaniana*	
Cyperceae	*Carex*	*rigescens*	
	Carex	*thomsonii*	
Elaeagnaceae	*Elaeagnus*	*bockii*	
	Elaeagnus	*pungens*	
Ericaceae	*Rhododendron*	*anthopogon*	
	Rhododendron	*bacetum*	
	Rhododendron	*capitatum*	
	Rhododendron	*clementinae*	
	Rhododendron	*concinnum*	*N*
Euphorbiaceae	*Euphorbia*	*helioscopia*	
Fagaceae	*Cyclobatonopsis*	*lamellosa*	
	Quercus	*acutissmia*	
	Quercus	*baronii*	
	Quercus	*variabilis*	
Gramineae	*Deyeuxia*	*scabrescens*	
	Fargesia	*spathacea*	
	Festuca	*subulata*	
	Imperata	*cylindrica*	
	Indocalamus	*scuriosis*	
	Pleioblastus	*amarus*	*N*
	Poa	*annua*	
	Poa	*nemoralis*	
	Roegneria	*kamoji*	
	Themeda	*triandra*	
Grossilariaceae	*Ribes*	*longiracemosum*	

FAMILY	GENUS	SPECIES	EATEN[a]
	Ribes	*meyeri*	
Helwingiaceae	*Helwingia*	*japonica*	
Hydrangeaceae	*Hydrangea*	*bretschneideri*	
Labiatae	*Phlomis*	*umbrosa*	*N*
Lardizabalaceae	*Akebia*	*trifoliata*	
Lauraceae	*Lindera*	*obtusiloba*	
	Litsea	*pungens*	
Liliaceae	*Asparagus*	*filicinus*	
	Fritillaria	*cirrhosa*	*N*
	Hemerocallis	*fulva*	
	Lilium	*brownii*	
	Notholirion	*hyacinthnum*	*N*
	Polygonatum	*odoratum*	
	Smilacina	*japonica*	
	Veratrum	*nigrum*	
Orchidaceae	*Bletilla*	*striata*	
Paeoniaceae	*Paeonia*	*lactiflora*	*N*
	Paeonia	*veitchii*	*N*
Papaveraceae	*Hylomecon*	*japonicum*	
Pinaceae	*Abies*	*shensiensis*	
	Abies	*sutchuenensis*	
	Tsuga	*chinensis*	
Pistaciaceae	*Pistacia*	*chinensis*	
Polygalaceae	*Polygala*	*arillata*	
	Polygala	*tenuifolia*	
	Rheum	*officnale*	
	Rheum	*palmatum*	
Polypodiaceae	*Pyrrosia*	*petiolosa*	
Ranunculaceae	*Aquilegia*	*lactiflora*	

FAMILY	GENUS	SPECIES	EATEN[a]
	Aquilegia	*yabeana*	
	Cimicifuga	*foetida*	
Rosaceae	*Agrimonia*	*pilosa*	
	Geum	*aleppican*	*N*
	Prunus	*tomentosa*	
	Pyracantha	*fortuneana*	
	Rosa	*hugonis*	
	Rosa	*multiflora*	
	Rubus	*corchorifolius*	
	Sorbaria	*arborea*	*N*
	Sorbus	*koehnei*	
	Spiraea	*alpina*	
	Spiraea	*rosthornii*	
Sabiaceae	*Sabia*	*shensiensis*	
Salicaceae	*Populus*	*davidiana*	
	Salix	*cuonlanis*	
	Salix	*glandulosa*	
	Salix	*pseudotangii*	
Saxifragaceae	*Astilbe*	*chinensis*	
	Rodgersia	*aesculifolia*	*N*
	Saxifraga	*stolonifera*	
Schisandraceae	*Schisandra*	*sphenanthera*	
Thymelaeaceae	*Daphne*	*giraldii*	
	Daphne	*tangutica*	
Tiliaceae	*Tilia*	*chinensis*	
Trilliaceae	*Paris*	*polyphylla*	
Umbelliferae	*Bupleurum*	*chinensis*	
	Daucus	*carota*	
	Ligusticum	*sinense*	*N*

FAMILY	GENUS	SPECIES	EATEN[a]
	Notopterygium	*forbesii*	
	Oenanthe	*javanica*	*N*
	Peucedanum	*decursivum*	
	Peucedanum	*praeruptorum*	
	Pleurospermum	*franchetianum*	*N*
	Sanicula	*chinensis*	*N*

a N indicates species not observed to be eaten by takins.

CHAPTER 3 RELATIONSHIP OF THE ASIAN TAKIN AND ARCTIC MUSKOX

INTRODUCTION

Sequencing of mitochondrial DNA (mtDNA) provides a modern tool for evaluating controversial aspects of phylogeny. Traditionally phylogenetic reconstruction has been based primarily on fossil records, morphology, ecology, and behavior as well as various molecular comparisons. Evidence of relationships from these different approaches can be contradictory, indicating some of these characteristics are weakly correlated with phylogeny or that they may evolve at different rates. The fundamental rate of evolution that has been documented for mtDNA (Irwin et al., 1991; Kraus and Miyamoto, 1991) provides an objective measure for comparison with other characteristics. This paper addresses the phylogeny of the takin (*Budorcas taxicolor*) and muskox (*Ovibos moschatus*), two Caprinae species hypothesized to be close relatives based on traditional phylogenetic characteristics.

Phylogenies of muskoxen and takins have long been debated. The muskox was first designated as *Bos moschatus* by Zimmerman (1780) based on descriptions of a strange buffalo-type animal encountered by early European explorers to North America. The genus *Ovibos,* which was proposed by Blainville in 1816 and had gained acceptance by the mid-1850's (Allen, 1913), indicates similarities of muskoxen to sheep and cows. The takin was first described by Hodgson (1850), who supposed it to be related to the "gnoo" or wildebeest (*Connochaetes taurinus*), but also suggested it was similar to the muskox. While Hodgson's name for the genus, *Budorcas*, stems from the Greek words for cow and gazelle, his detailed descriptions repeatedly refer to similarities to both sheep and goats.

Matschie (1898) believed takins were closely related to muskoxen and proposed a subfamily, Ovibovinae, consisting of only these two species. In a classification of Artiodactyla, Knotterus-Meyer (1907) proposed a family, Obovidae, which contained the

genera *Ovibos*, *Budorcas* and *Connochaetes*. More recently, Simpson (1945) classified the takin and muskox within the Ovibovini, one of four tribes in the subfamily Caprinae. Serological studies by Moody (1958) and restriction-site mapping of ribosomal DNA by Wall et al. (1992) established muskoxen as being more similar to sheep and goats than to cattle and bison, thus supporting placement of at least *Ovibos* within the Caprinae.

Close evolutionary relationships between the takin and muskox have been supported by fossil, morphological, ecological, and chromosomal evidence. Paleontological evidence indicates the genus *Boopsis,* which may be similar to an ancestral form of muskox, appeared in Asia in the Lower Pliocene (Crégut-Bonnoure, 1984). Pleistocene fossils of primitive *Ovibos* occur across Europe, Asia and North America (McDonald and Davis, 1989). Early fossils of takin (*B. teilhardi*) date from the upper Pliocene in China (Neas and Hoffmann, 1987). No fossil form ancestral to both the takin and muskox has been identified, but Harington (1989) proposed *Budorcas* to be related to the primitive muskox genera *Boopsis* and *Soergelia*.

Morphological criteria for relatedness in Caprinae center on body size and horn characteristics. Among Caprinae, the takin and muskox are distinct because of their large body size ($\geq$ 300 kg for males) as opposed to 50 to 150 kg for most other Caprinae (Schaller, 1977). Adults of both species possess horns that originate between the orbits and occipital plane. Takin horns, which are black and transversely-ringed, are vertical at the base but turn outward and curve up and back to a point. The whitish horns of the muskox, which are longitudinally-striated, curve down along the side of the face, and then up and out to a point. Males of both species use their horn bosses during dominance fights, whereas both sexes use the pointed horn tips as defense against predators (Tener, 1965; Wu, 1990). Lander (1919), in comparing the soft anatomy of the takin and muskoxen, reported many similarities that she believed differentiated these ungulates from other species. Lönnberg (1900b), on the other hand, thought resemblance between the species was only superficial.

Comparative cytogenetics has been prominent in phylogenetic reconstructions because of the implications of changes in chromosome structure in altering the genetic code. The fundamental number (FN) of chromosomes of both the takin and muskox is

60, although the modal diploid (2n) of the takin is 52 (Bogart and Benirschke, 1975) and that of the muskox is 48 (Tietz and Teal, 1967). The difference in 2n between the species could be explained by Robertsonian rearrangements and does not preclude the possibility of a close relationship (Bogart and Benirschke, 1975). When high-resolution G-banding was used to compare chromosomes of the takin and muskox, morphological similarities and homology of banding patterns were observed (Pasitschniak-Arts et al., 1994), which the authors believe supports a close relationship between these species.

While many aspects of social ecology change with habitat, evidence of similarities in behavior and ecology may be used to deduce relatedness. Among ungulates, group size, mating systems, reproductive behavior of females, and strategies for predator avoidance (Jarman and Jarman, 1979; Lott, 1991) all tend to vary with habitat. Muskoxen inhabit treeless tundra, whereas most takins frequent steep, heavily-vegetated mountains at temperate latitudes. Despite occupying vastly different habitats, the takin and muskox share similarities in social ecology: both species commonly live in mixed-sex groups, are harem breeders, their calves are followers rather than hiders, and both utilize group defense against predators (Lent, 1974; Gray, 1987; Wu, 1990). Considering the diversity of social ecology within the Caprinae (Schaller, 1977), these similarities could be viewed as an indication of a common phylogenetic history for the takin and muskox.

Despite apparent phylogenetic similarities between the takin and muskox, the relationship between the species has not been firmly established. Comparison of mtDNA from these species could serve to define their relationship. MtDNA is useful for studying the phylogeny of animals because it is small relative to nuclear DNA, is maternally inherited (Giles et al., 1980) with no recombination, and has a rate of evolution about 10 times higher than single copy nuclear DNA (Brown et al., 1982). The cytochrome *b* gene is useful for analyzing interspecific phylogenies because the conservative nature of a protein-coding gene allows one set of primers to work over a variety of taxa (Kocher et al., 1989) and facilitates alignment of sequences.

We used sequence data to test the long-standing hypothesis that the takin and muskox are sister taxa. Cytochrome *b* sequence was analyzed for both the takin and

muskox as well as for one species from each of the other three Caprinae tribes (Simpson, 1945): saiga (*Saiga tatarica)* from the Saigini, Chinese goral (*Nemorhaedus goral)* from the Rupicaprini and bighorn sheep (*Ovis canadensis)* from the Caprini. Comparison of DNA sequences of these species provides a framework for interpretation of the relationships between the takin and muskox.

MATERIALS AND METHODS

MtDNA sequences were generated for five species of Caprinae (Table 3 - 1). The published sequence of domestic cow (Bovinae) was included in the analysis as an outgroup. DNA was extracted from 300 μl aliquots of whole blood by differentially lysing red and white blood cells, precipitating residual proteins with a high salt solution, and finally precipitating the DNA with isopropanol. DNA was extracted from 20-μg sections of frozen muscle, heart, liver, spleen, placenta, and dried skin using standard lysis and digestion protocols (Maniatis et al., 1982) followed by salt and isopropanol precipitations.

Portions of the mitochondrial cytochrome *b* gene were amplified from all samples via PCR using four pairs of primers (Table 3 - 2). Most amplifications were 50 μl asymmetric reactions with primer ratios between 1:10 and 1:100, which generated single-stranded DNA for sequencing (Gyllensten and Erlich, 1988). Some samples required a double-stranded amplification with primers in equal concentrations followed by a single-stranded amplification with only one primer (Kocher et al., 1989). Amplified DNA was visualized by ethidium-bromide staining of agarose gels. Amplification products in the predicted size range were purified by three centrifugal filtrations with 350 μl of water in Ultrafree MC 30,000 NMWL (Millipore) tubes. DNA was resuspended in 25 μi sterile water and sequenced using the dideoxy sequencing methods of Sanger et al. (1977). Specifically, 7 μl of the PCR product, the limiting primer in the PCR reaction, and Sequenase Version 2.0 DNA Sequencing kits (U.S. Biochemical) were used, following protocols described with the kit. Because primer pairs over-lapped sequentially, continuous sequence was obtained for most individuals by sequencing in only one direction. Individuals that were sequenced in both directions all yielded complementary sequences. Sequences were read with the help of DNA Parrot DP

Table 3 - 1. Species, location[a], type, number (N), source of tissues or sequence analyzed, and number of base pairs sequenced (BP) for all taxa included in analysis.

Species	Common name	Location	Tissue	N	Source	# BP
Ovibos moschatus moschatus	Barren-ground muskox	Coppermine, NWT	Blood	5	Anne Gunn,. Renewable Resources, Government Northwest Territories(GNWT)	
"	"	Rendezvous Lake, NWT	Muscle	5	Renewable Resources, GNWT	
Ovibos moschatus wardi	White-faced muskox	Banks Island, NWT	Blood, heart	9	Renewable Resources, GNWT	
"	"	Victoria Island, NWT	Blood	5	Anne Gunn, Renewable Resources, GNWT	
"	"	West Greenland	Muscle	2	Greenland Environmental Research Institute, Copenhagen	
"	"	Fairbanks, AK	Blood, skin	6	Large Animal Research Station, University of Alaska Fairbanks	
"	"	Palmer, AK	Blood, placenta	6	Musk Ox Development Corporation	
			Total	**38**		**1,044**
Budorcas taxicolor bedfordi	Golden takin	Shaanxi, China	Skin	2	Shaanxi Institute of Zoology	
"	"	Apple Valley, MN	Blood	1	Nick Reindl, Minnesota Zoo	
"	"	San Diego, CA	Blood	1	Oliver Ryder, San Diego Zoo	
Budorcas taxicolor tibetana	Sichuan takin	San Diego, CA	Blood	2	Oliver Ryder, San Diego Zoo	
Budorcas taxicolor taxicolor	Mishmi takin	Berlin Zoo	Blood	2	Oliver Ryder, San Diego Zoo	
			Total	**8**		**1,170**
Nemorhaedus caudatus	Chinese goral	San Diego, CA	Liver	1	Oliver Ryder, San Diego Zoo	1,187
Saiga tatarica	Saiga	San Diego, CA	Liver	1	Oliver Ryder, San Diego Zoo	1,003
Ovis canadensis	Bighorn sheep	Alberta	Muscle	1	Curtis Strobeck, University of Alberta	846
Bos taurus	Domestic cow		Sequence	1	Irwin et al., 1991	1,140

[a] For takin and muskox distribution and location, see Figs. 1 and 2.

Table 3 - 2. Primers used to amplify and sequence cytochrome *b* gene. L and H refer to light and heavy strands, respectively. Numbering is from the 3' end and is based on the system of Anderson et al. (1981).

Name of primer	Sequence
L 14724[a]	5' CGAAGCTTGATATGAAAAACCATCGTTG 3'
L 14841[b]	5' AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA 3'
L 15069	5' GCCTATACTACGGATCATACAC 3'
H 15149[b]	5' AACTGCAGCCCCTCAGAATGATATTGTCCTCA 3'
L 15275	5' GACAAAGCATCCCTCACCCG 3'
H 15338	5' CTGTTTCGTCCACCAAGAG 3'
L 15513[a]	5' CTAGGAGACCCTGACAACTA 3'
H 15608	5' TAGGCTAGAACTCCGCCTAG 3'
H 15915[a]	5' AACTGCAGTCATCTCCGGTTTACAAGAC 3'

a Primers described by Irwin et al. (1991).
b Primers described by Kocher et al. (1989).

100-PC™ (Clontech) and aligned using Align Plus Version 2 (Scientific and Educational Software). Analysis of sequences was based on 1,140 base pairs of the cytochrome *b* gene as described by Irwin et al. (1991). Phylogenetic analysis was conducted using PAUP Version 3.1 (Swofford, 1993) for parsimony analysis and PHYLIP Version 3.53 (Felsenstein, 1993) for distance and maximum likelihood analysis.

RESULTS

A mean of 1,044 (Range = 941 - 1,164) nucleotides of the cytochrome *b* gene were obtained for 38 muskoxen from two subspecies (Fig. 3 - 1). There was variation at five sites among these animals, but only one site was variable in more than one individual. This transversion site was treated as a polymorphism in the parsimony analysis.

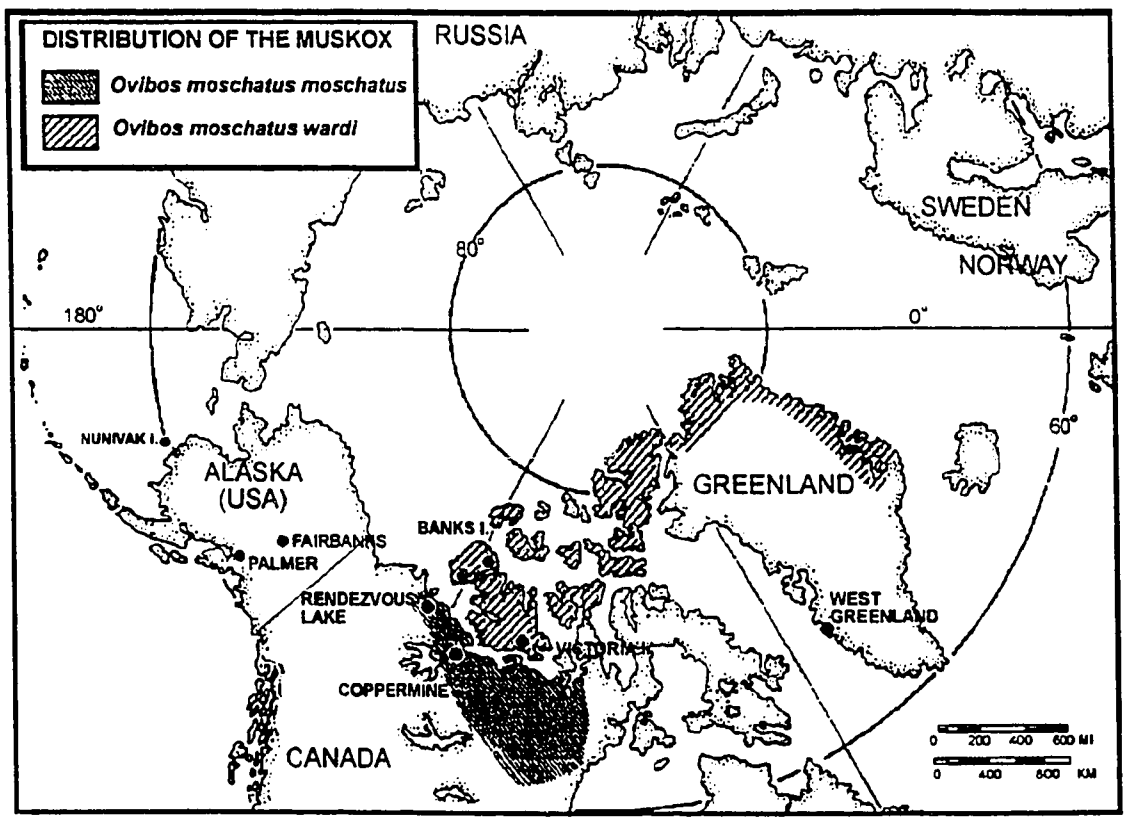

Figure 3 - 1. Distribution of muskox subspecies. Closed circles represent locations from which samples were obtained. Muskoxen within Alaska are *O. m. wardi.*

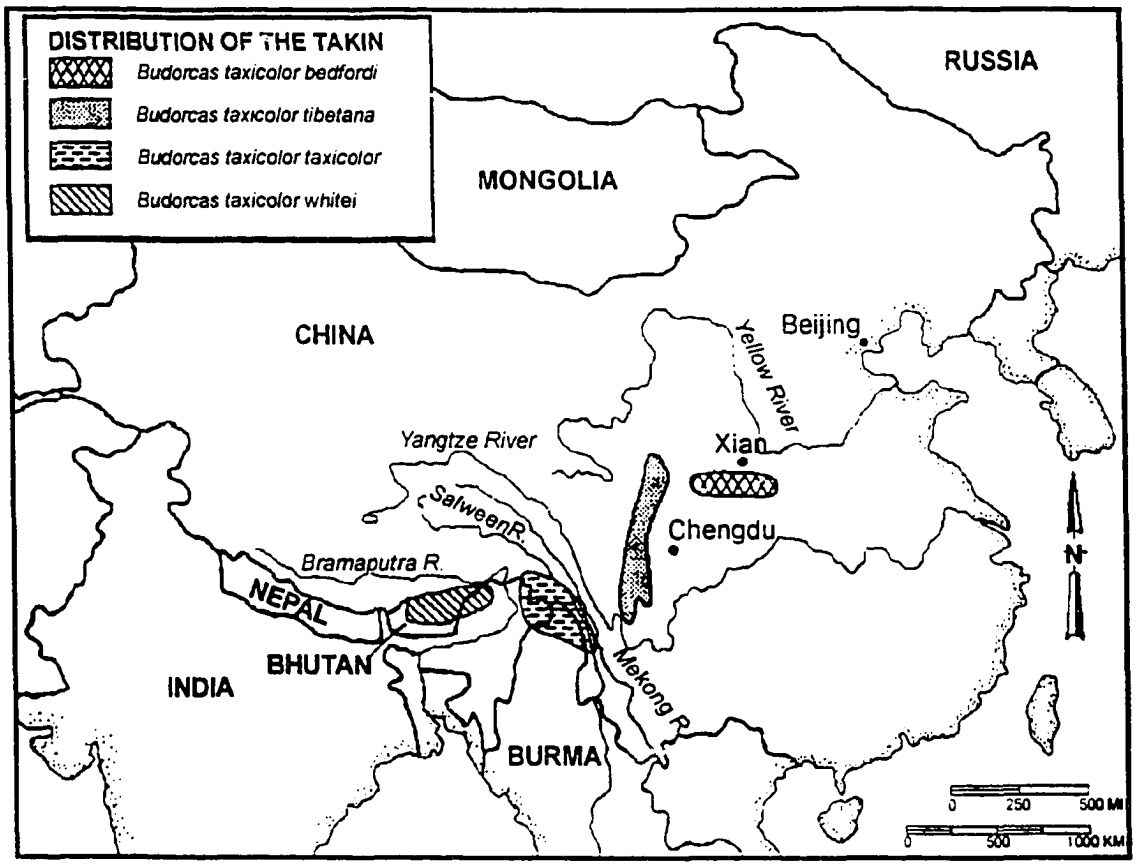

Figure 3 - 2. Distribution of takin subspecies. Individuals from *B. t. bedfordi*, *tibetana*, and *taxicolor* were included in this study.

Eight takins from three subspecies (Fig. 3 - 2) all yielded sequences of 1,170 base pairs encompassing the entire cytochrome *b* gene. Sequences for all individuals of *B. t. bedfordi* and *B. t. tibetana* were identical. The two sequences from *B. t. taxicolor* were identical and differed from those of the other takins at nine sites. These variations, which included six third-position transitions, two first-position transitions, and one third-position transversion, were treated as polymorphisms in the parsimony analysis.

DNA of only one bighorn sheep, goral and saiga antelope each was sequenced. The amount of sequence obtained for these species was 1,003, 1,187, and 846 base pairs, respectively. No deletions or insertions were observed, which facilitated alignment of all sequences. Among the sites compared between all six taxa, 329 substitutions were observed of which 138 were phylogenetically informative with the change observed in more than one taxon. These informative changes included six first-position, 17 third-position transversions, 23 first-position, eight second-position, and 84 third-position transitions. The 1,140 base-pair sequences had predicted translation products of 379 amino acids. DNA of all taxa terminated with an AGA stop codon.

Pairwise comparisons

Pairwise comparisons of sequence differences between taxa can provide a simple estimate of evolutionary distance. The sequence divergence based on all substitutions among the five Caprinae species ranged from 6.0 to 14.9% and based on transversions only between 1.2 and 4.6% (Table 3 - 3). Divergences between the muskox and takin, 11.8% for all substitutions, 2.1% for transversions only, and 4.7% for third-position transversions, were larger than divergences between the muskox and goral or the takin and bighorn sheep. Pairwise transition:transversion ratios should decrease with increasing distance between taxa as transition sites become saturated (Brown et al., 1982). The highest transition:transversion ratio was between the muskox and goral, not between the muskox and the takin (Table 3 - 3). These values suggest that the muskox and takin are not sister taxa.

Table 3 - 3. Pairwise comparisons between taxa based on 1,140 bp of the cytochrome *b* gene.

		Percentage divergence based on all substitutions (above diagonal). Transition:transversion ratios (below diagonal).						Percentage divergence based on transversions. Third positions of codons only (above diagonal). All codon positions (below diagonal).					
	Species	1	2	3	4	5	6	1	2	3	4	5	6
1	Muskox		11.3	11.0	11.8	14.9	14.1		2.3	4.9	4.7	7.9	11.1
2	Goral	8.21		11.9	12.4	14.9	16.4	1.2		4.6	4.0	8.2	10.3
3	Bighorn sheep	3.50	3.88		6.0	13.2	15.3	2.4	2.4		2.1	9.3	10.1
4	Takin	4.96	5.82	3.53		14.4	16.1	2.1	1.9	1.5		8.5	10.0
5	Saiga	2.44	2.28	1.89	2.40		16.7	4.3	4.5	4.6	4.4		10.1
6	Cow	2.16	2.98	2.33	2.82	2.16		4.5	4.1	4.6	4.5	5.3	

Maximum parsimony

A single, most-parsimonious tree resulted when all 138 phylogenetically informative sites were included in the analysis, and cow was included as an outgroup (Fig. 3 - 3). This tree had a length of 257 mutational steps and a consistency index of 0.63. The g_1 statistic for this tree was -0.77 indicating a significant skew to the tree-length distributions and therefore a strong phylogenetic signal in these data (Hillis and Huelsenbeck, 1992). Because transitions accumulate more rapidly than transversions, especially at third-positions of codons (Brown et al., 1982), parsimony may be more accurate if some or all transition sites are ignored. Parsimony trees were generated excluding all third-position transitions and using transversion sites only. A single most parsimonious tree was generated from each of these approaches. Both of these trees had the same topology as the first tree and had lengths of 96 and 40, and consistency indices of 0.70 and 0.75, respectively. The g_1 statistics for these trees (-1.01 and -0.89) both indicated strong phylogenetic signal as well. All three approaches to maximum-parsimony analysis strongly separate the muskox and takin into two separate clades, each with another more closely related species.

Distance

To evaluate the relationship between the takin and muskox from a different perspective, the neighbor-joining distance method from PHYLIP 3.53 (Felsenstein, 1993) was used. Distances used in this tree were calculated using the Kimura two-parameter model, which is based on different rates for transitions and transversions. The topology of this tree was identical to those generated using parsimony methods (Fig. 3 - 3), further supporting the evolutionary distance between the takin and muskox.

Maximum likelihood

A maximum-likelihood tree was estimated with DNAML in PHYLIP 3.53 (Felsenstein, 1993), using the observed nucleotide frequencies and a transition:transversion ratio of 3.6, which maximized the likelihood. Topology of this tree was identical to trees based on parsimony and distance analyses with the takin and muskox separated into different clades.

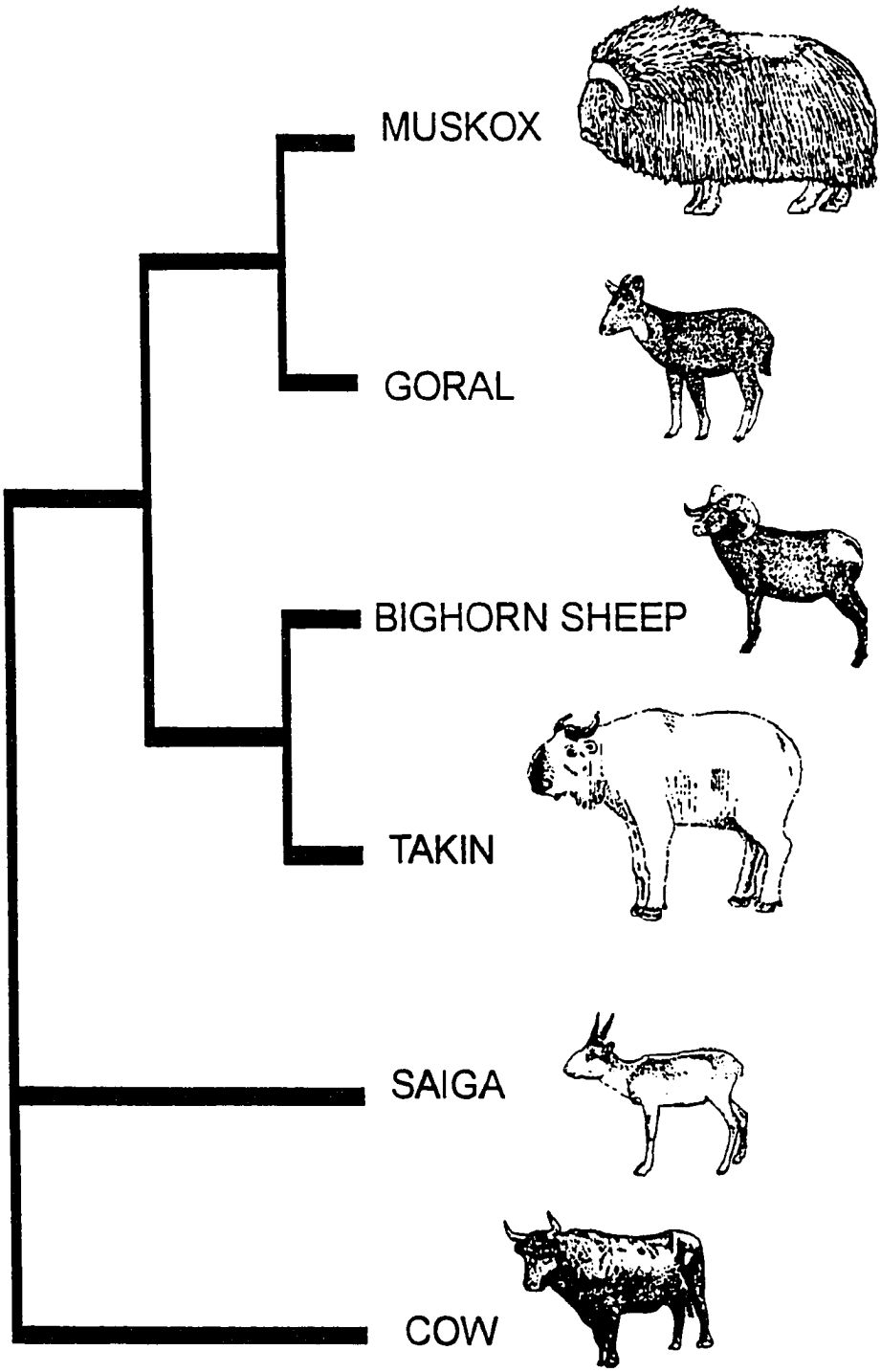

Figure 3 - 3. Phylogenetic gene tree of cytochrome *b* gene of mitochondrial DNA showing relationships of five Caprinae species. This same topology was obtained by maximum parsimony, distance and maximum likelihood methods. Cow was included as an outgroup. Drawings are not to scale.

The log likelihood of different tree topologies can be calculated using DNAML. Tree topologies that forced the takin and muskox into the same clade were all lower in log likelihood than the topology that separated the two species. The best of these alternative topologies, which placed the muskox, takin and goral into one clade, was lower in log likelihood by 36.4 $\pm$ 13.6 (calculated using formula of Kishino and Hasegawa, 1989, in DNAML) and therefore significantly worse than the topology of Fig. 3 - 3. This result further supports the evidence for phylogenetic separation between the takin and muskox.

The tree generated using DNAMLK (Felsenstein, 1993), which assumes a molecular clock, or constant rate of evolution among lineages, had the same topology as the DNAML tree. Therefore, rate constancy could be tested with a standard likelihood-ratio test. The degrees of freedom were n - 2 where n equals the number of taxa. Twice the difference of the log likelihoods was 6.82. On a chi-square distribution ($d.f.$ = 4, $P < 0.05$) the critical value is 9.49. Because this test value was not significant, a constant rate of evolution was supported.

With the molecular clock thus supported, estimates of times of divergence between taxa can be made. Within mammalian mtDNA, sequence divergence of transversions is linearly correlated with time (Miyamoto and Boyle, 1989). The transversion divergence rate for third positions of codons of the cytochrome *b* gene has been calculated at 0.5% per million years (Irwin et al., 1991). Based on this estimate, the takin and muskox would have diverged approximately 9.4 million years ago, while the takin and bighorn sheep would have diverged 4.2 million years ago and the muskox and goral 4.6 million years ago (Fig. 3 - 3).

DISCUSSION

Analysis of cytochrome *b* sequences indicates that the takin and muskox both belong within the Caprinae, but are not close relatives; each species is more closely related to other species within this subfamily. We suggest the Ovibovini proposed by Simpson (1945) is not a valid tribe and the takin belongs in the Caprini, whereas the muskox belongs in the Rupicaprini. Comparisons with other species are required to establish more specific relationships within the Caprinae. Lack of a close relationship between the takin and muskox, despite morphological and chromosome similarities, is

not surprising when the confusing phylogeny of the Caprinae and the entire Bovidae is considered (Schaller, 1977; Geist, 1987; Gatesy et al., 1992). Since the Bovidae first appeared during the Miocene, rapid radiation has resulted in a diversity of species, both extinct and extant (Savage and Russell, 1983). With such high diversity, convergent evolution can be expected to have resulted in similarities between distantly related species.

Whereas the fossil history for much of the Bovidae is well documented, that of the Caprinae is less clear. This is largely due to the mountainous environment in which the Caprinae evolved not supporting a rich fossil history (Simpson, 1945). Ancestral muskoxen had a wide distribution across Europe, Asia, and North America and were not restricted to mountainous habitats (Crégut-Bonnoure, 1984), consequently they have left a fossil record. Fossil history for takins is more sparse than that for muskoxen (Neas and Hoffmann, 1987), and a common takin-muskox ancestor in the fossil record only has been hypothesized, never identified.

A morphological feature that appears to unite the takin and muskox, and to separate the takin from sheep, is the number of mammae. The takin and muskox both have two pairs of mammae whereas sheep and most Caprini species have only one pair. Species within other tribes of the Caprinae all have two pairs. Among bovids, most species have two pairs of mammae. Exceptions are Alcelaphini (gnus etc.), Antilopini (springbok, *Antidorcas marsupialis*) and Caprini (Smithers, 1983; Nowak, 1991). Significance of the number of mammae is not clearly understood, but the number appears to be plastic within the Bovidae. Among springbok, most females have one pair, but females with two pairs are not uncommon (Smithers, 1983). Within the genus *Hemitragus* (tahr) in the Caprini, one species has one pair, while the other two species have two pairs of mammae (Nowak, 1991). This variation at an interspecific and intergeneric level indicates the number of mammae is probably not an informative phylogenetic character.

The distinctive horns of the takin and muskox have commonly been used to relate these species. No other Caprinae have similar horns, but within the Bovidae, the wildebeest and African buffalo (*Syncerus caffer*) do. The males of all four of these species use their horns during dominance fights. The takin, muskox, and African

buffalo all use their horns to defend the group against predators. That such divergent species all have similar horns, we believe, indicates that horn shape is a plastic morphological feature more strongly tied to ecology and body size than to phylogeny for this group.

Body size is another character that distinguishes the takin and muskox from other Caprinae. Increased body size confers advantages such as improved ability to withstand temperature extremes, lower mass-specific metabolic rate, ability to digest lower-quality food, and ability to repulse predators (Kleiber, 1975; Schaller, 1977; Hudson, 1985; Hofmann, 1989). Because of these advantages, we believe large body size can be expected to evolve independently in separate lineages, especially within a group as diverse as the Caprinae. For muskoxen living in the Arctic, large size is adaptive for the cold climate, consuming a low-quality winter diet, and avoiding predation. The takin does not live in such an extreme climate as the muskox, but is confronted with much more interspecific competition for resources (Wu, 1990). Large size allows the takin to consume food that is unavailable to the smaller ungulates in that habitat, either because of lower quality or physical inaccessibility, as well as to avoid predation.

We propose similarities in social ecology between the takin and muskox can be attributed to shared body size and horn shape rather than shared ancestry. Both species attempt to repulse predators rather than run from them. Group defense against predators is common only among large-bodied species with stout horns (i.e. takin, muskox, and African buffalo; Sinclair, 1977), but is only practical if the species lives as a group. Sexual dimorphism among the takin and muskox is not as pronounced as among many of the other Caprinae (Schaller, 1977). Males and females can consume similar generalist diets and remain in mixed-sex groups throughout the year. Because both species rely on group defense, their calves are more likely to avoid predation as followers rather than hiders.

Although the takin and muskox have different diploid numbers of chromosomes, some authors have observed similarities between the chromosomes of the two species, which they believe support the close relationship of the species (Bogart and Benirschke, 1975; Pasitschniak-Arts et al., 1994). These comparisons however, did not

include other Caprinae species to evaluate whether more similarities might occur between these other species. Within the Caprinae, 2n ranges from 42 to 60; with bighorn sheep having 2n = 54 (Nadler et al., 1973) and goral 2n = 56 (Soma et al., 1987). Without pairwise comparisons to more Caprinae karyotypes, chromosomal evidence cannot be used to define the relationship between the takin and muskox. G-band patterns in some chromosomes of turtles (Cryptodira) have been conserved for 200 million years of divergent evolution (Bickham, 1981), so similarities of these patterns is not the most convincing argument for close relationship.

Traditional phylogenetic characteristics may not reconstruct phylogeny as accurately as molecular characteristics accessible through modern techniques. Linguistic affiliation has long been used to relate human populations. Recent mtDNA studies on northern North American populations have revealed incongruencies between linguistic and genetic divergence indicating that language can evolve more rapidly than genes (Shields et al., 1993; Ward et al., 1993). Some morphological characteristics appear similar to language in the ability to change more rapidly than the underlying genetic sequence, especially when under strong selective pressure.

The comparison of cytochrome *b* sequences of the takin and muskox has provided a new perspective on the relationship of these species. Based on the assumption of a molecular clock, the two species have been separated over 9 million years, whereas each may have diverged from another species less than 5 million years ago. The paucity of Caprinae fossils has prevented calibration of the rate of evolution for this subfamily, but while time estimates may not be exact, the relative times are likely valid. Clearly, similarities between the species cannot be attributed to their common phylogeny and shared genetic heritage, but demonstrate the force of natural selection and convergent evolution in selecting for the development of similar characteristics in separate lineages. These characteristics must be plastic and able to change within the constraints of the genetic heritage. Advantages of large body size and horns effective as predator defense are significant enough that these combined characteristics can be expected to evolve independently as lineages adapt to different environments. Because of the interrelationship of morphological and ecological characteristics, a suite of similarities can develop between species that are not

reflective of close genetic ancestry, but have evolved in response to similar types of ecological pressures.

CHAPTER 4 PHYLOGENETICS OF THE CAPRINAE BASED ON CYTOCHROME *b* SEQUENCE



INTRODUCTION

The subfamily Caprinae of the family Bovidae consists of sheep, goats and related species that mostly live in remote, mountainous habitats. Relationships within this subfamily, as within much of the Bovidae, have never been fully resolved. The Bovidae first appeared about 20 million years ago (MYA) and subsequently underwent rapid radiation that resulted in extreme diversity and wide distribution; this pattern of speciation has complicated classification (Gatesy et al., 1992). Classification and the number of species within the Caprinae is still being debated, but the divisions suggested by Simpson (1945) and Nowak (1991) have been widely accepted (Table 4 - 1). Unlike most Bovidae, Caprinae have a relatively poor fossil record due to the mountainous habitats in which they evolved (Simpson, 1945). Consequently, phylogenetic classification has been based primarily on morphology, ecology, behavior and, most recently, molecular comparisons.

Four tribes have been recognized within the Caprinae. The tribe Saigini is divergent enough from other Caprinae that some exclude it from the subfamily (Geist, 1987). The Ovibovini are distinct in that the two species are the largest Caprinae, but debate has frequently surrounded the hypothesized relationship of the muskox (*Ovibos moschatus*) and takin (*Budorcas taxicolor*), which has been based on morphological and behavioral similarities (Neas and Hoffmann, 1987).

Because of their morphology and behavior, the Rupicaprini have been hypothesized to be the ancestral group of Caprinae from which the Ovibovini and Caprini evolved (Geist, 1971; Schaller, 1977). Recent studies comparing isoenzymes and allozymes of some species in these groups have contradicted this accepted pattern of Caprinae phylogeny (Hartl et al., 1990; Randi et al., 1991). Randi et al. (1991) proposed that the genera *Rupicapra* within Rupicaprini, and *Capra* (goats) and

Table 4 - 1. Caprinae classification[a].

Genus	Species	Common Name	2n[b]
Tribe SAIGINI			
Pantholops	*hodgsoni*	Chiru (Tibetan antelope)	
Saiga	***tatarica***	**Saiga**	**60**
Tribe RUPICAPRINI			
Nemorhaedus	***caudatus***	**Chinese goral**	**56**
	goral	Himalayan goral	56
	baileyi	Red goral	56
Capricornis	***crispus***	**Japanese serow**	**50**
	sumatraensis	Sumatra serow	48
	swinhoei	Formosa serow	50
Oreamnos	***americanus***	**Mountain goat**	**42**
Rupicapra	*rupicapra*	Chamois	58
	pyrenaica	Chamois	58
Tribe OVIBOVINI			
Ovibos	***moschatus***	**Muskox**	**48**
Budorcas	***taxicolor***	**Takin**	**52**
Tribe CAPRINI			
Ammotragus	*lervia*	Aoudad (Barbary sheep)	58
Pseudois	*nayaur*	Bharal (Blue sheep)	54
Hemitragus	***jemlahicus***	**Himalayan tahr**	**48**
	hylocrius	Nilgiri tahr	
	jayakeri	Arabian tahr	
Capra	***hircus***	**Domestic goat**	**60**
	aegagrus	Wild goat	60
	ibex	Ibex	60
	walie	Walia ibex	
	caucasia	West Caucasian tur	
	cylindricornis	East Caucasian tur	
	pyrenaica	Spanish ibex	
	falconeri	Markhor	60
Ovis	***aries***	**Domestic sheep**	**54**
	canadensis	**Bighorn sheep**	**54**
	dalli	**Dall's sheep**	**54**
	vignei	Urial	58
	ammon	Argali	56
	orientalis	Asiatic mouflon	54
	musimon	Mouflon	54
	nivicola	Snow sheep	52

Note: Species included in this study indicated in boldface.

[a] Based on Simpson (1945) and Nowak (1991).

[b] Diploid chromosome number.

Ovis (sheep) within Caprini are all equidistant from each other and evolved from a common contemporaneous ancestor. Hartl et al. (1990) reported *Capra* and *Ovis* are more divergent from each other than either is from *Rupicapra*, suggesting the tribal separation of Rupicaprini and Caprini is not valid.

The availability of data from mitochondrial DNA (mtDNA) sequences has provided new perspectives on previously confusing questions of phylogeny. The cytochrome *b* gene in the mitochondrial genome is useful for analyzing interspecific relationships because the conservative nature of a protein-coding gene facilitates alignment of sequences between taxa. The rate of evolution of this gene which has been documented (Irwin et al., 1991) is appropriate for investigating events that have occurred within the last 20 million years such as the evolution of the Caprinae. We chose to use cytochrome *b* sequence data to attempt to clarify some relationships within the Caprinae.

METHODS

MtDNA sequences were generated for nine Caprinae species during this study (Table 4 - 2). Sequence analysis included published sequences of two additional Caprinae as well as domestic cow (*Bos taurus*), a Bovinae, for an outgroup (Table 4 - 2).

DNA was extracted from 300 μl aliquots of whole blood by differentially lysing red and then white blood cells, precipitating residual proteins with high salt and finally precipitating the DNA with isopropanol using protocols adapted from Miller et al. (1988) and Winberg (1991). DNA was extracted from 20 μg sections of frozen muscle, heart, liver, spleen, placenta and dried skin using standard lysis and digestion protocols (Maniatis et al., 1982) followed by salt and isopropanol precipitations. Purified DNA of the Japanese serow (*Capricornis crispus*) was obtained (Table 4 - 2), so no extractions were conducted on that sample.

Portions of the mitochondrial cytochrome *b* gene were amplified from all samples via the polymerase chain reaction (PCR) using four pairs of primers (Table 4 - 3). Most amplifications were 50 μl asymmetric reactions with primer ratios between 1:10 and 1:100, which generated single-stranded DNA for sequencing (Gyllensten and

Table 4 - 2. Species, location, type, number, number of base pairs sequenced and source of tissue analyzed.

Species	Common Name	Location	Tissue Type	N	# BP	Source
Ovibos moschatus moschatus	Barren-ground muskox	Coppermine, NWT	Blood	5		Dept. Renewable Resources, Government Northwest Territories (GNWT)
"	"	Rendezvous Lake, NWT	Muscle	5		Dept. Renewable Resources, GNWT
Ovibos moschatus wardi	White-faced muskox	Banks Island, NWT	Blood, Heart	9		Dept. Renewable Resources, GNWT
"	"	Victoria Island, NWT	Blood	5		Dept. Renewable Resources, GNWT
"	"	West Greenland	Muscle	2		Greenland Environmental Research Institute, Copenhagen
"	"	Fairbanks, AK	Blood, Skin	5		Large Animal Research Station, University of Alaska Fairbanks
"	"	Palmer, AK	Blood, Placenta	6		Musk Ox Development Corporation
"	"	Nunivak Island, AK	Muscle	1		Hunter killed
			Total	***38***	**1,140**	
Budorcas taxicolor bedfordi	Golden takin	Shaanxi, China	Skin	2		Shaanxi Institute of Zoology
"	"	Apple Valley, MN	Blood	1		Nick Reindl, Minnesota Zoo
"	"	San Diego, CA	Blood	1		Oliver Ryder, San Diego Zoo
Budorcas taxicolor tibetana	Sichuan takin	San Diego, CA	Blood	2		Oliver Ryder, San Diego Zoo
Budorcas taxicolor taxicolor	Mishmi takin	Berlin Zoo	Blood	2		Oliver Ryder, San Diego Zoo
			Total	***8***	**1,140**	
Capricornis crispus	Japanese serow	San Diego, CA	DNA	1	955	Oliver Ryder, San Diego Zoo
Nemorhaedus caudatus	Chinese goral	San Diego, CA	Liver	1	1,140	Oliver Ryder, San Diego Zoo
Hemitragus jemlahicus	Himalayan tahr	San Diego, CA	Spleen	1	1,004	Oliver Ryder, San Diego Zoo
Saiga tatarica	Saiga	San Diego, CA	Liver	1	947	Oliver Ryder, San Diego Zoo
Oreamnos americanus	Mountain goat	Alberta	Muscle	1	964	Curtis Strobeck, University of Alberta
Ovis canadensis	Bighorn sheep	Alberta	Muscle	1	983	Curtis Strobeck, University of Alberta
Ovis dalli	Dall's sheep	Alaska	Muscle	1	969	Greg Finstad, Fairbanks, AK
Ovis aries	Domestic sheep		Sequence	1	1,140	Irwin *et al.*, 1991
Capra hircus	Domestic goat		Sequence	1	1,140	Irwin *et al.*, 1991
Bos taurus	Domestic cow		Sequence	1	1,140	Irwin *et al.*, 1991

Erlich, 1988). Some samples required a double-stranded amplification with primers in equal concentrations followed by a single-stranded amplification with only one primer(Kocher et al., 1989). Amplified DNA was visualized by ethidium bromide staining of agarose gels. Amplifications in the predicted size range were purified by three centrifugal filtrations with 350 µl of water in Ultrafree MC 30,000 NMWL (Millipore) tubes. DNA was resuspended in 25 µl sterile water and sequenced using the dideoxy sequencing methods of Sanger et al. (1977). Specifically, 7 µl of the PCR product, the

Table 4 - 3. Primers used to amplify and sequence cytochrome *b* gene.

Name of Primer	Sequence
L 14724[a]	5' CGAAGCTTGATATGAAAAACCATCGTTG 3'
L 14841[b]	5' AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA 3'
L 15069	5' GCCTATACTACGGATCATACAC 3'
H 15149[b]	5' AACTGCAGCCCCTCAGAATGATATTGTCCTCA 3'
L 15275	5' GACAAAGCATCCCTCACCCG 3'
H 15338	5' CTGTTTCGTCCACCAAGAG 3'
L 15513[a]	5' CTAGGAGACCCTGACAACTA 3'
H 15608	5' TAGGCTAGAACTCCGCCTAG 3'
H 15915[a]	5' AACTGCAGTCATCTCCGGTTTACAAGAC 3'

L and H refer to light and heavy strands, respectively. Numbering is from the 3' end and is based on the system of Anderson et al. (1981).
a Primers described by Irwin et al. (1991).
b Primers described by Kocher et al. (1989).

limiting primer in the PCR reaction and Sequenase Version 2.0 DNA Sequencing kits (U.S. Biochemical) were used, following protocols described with the kit. Because the primer pairs over-lapped sequentially, continuous sequence was obtained for most individuals by sequencing in only one direction. Samples that were sequenced in both directions yielded complementary sequences. Sequences were read with the help of DNA Parrot DP 100-PC™ (Clontech) and aligned using Align Plus Version 2 (Scientific and Educational Software). Analysis of the sequences was based on the 1,140 bp of the cytochrome *b* gene as described by Irwin et al. (1991). Pairwise and base

composition comparisons were made using MEGA (Kumar et al., 1993). Phylogenetic analysis was conducted using the PAUP Version 3.1 (Swofford, 1993) branch and bound algorithm for maximum parsimony analysis and PHYLIP Version 3.5 (Felsenstein, 1993) for distance and maximum-likelihood analysis.

RESULTS

The number of base pairs included in the analysis varied among species (Table 4 - 2). Among 38 muskoxen, variation occurred at five sites, but only one site was variable in more than one individual. This transversion was treated as a polymorphism in parsimony analysis and the consensus sequence was used in distance and maximum-likelihood analyses. Among the eight takins, sequences for all *B. t. bedfordi* and *B. t. tibetana* individuals were identical. The two *B. t. taxicolor* sequences were identical and differed from those of the other takins at nine sites. These variations which included six third-position transitions, two first-position transitions and one third-position transversion, were treated in the same manner as for muskoxen.

Among the 1,140 sites compared between all 12 taxa, 396 substitutions were observed; 233 of those substitutions were phylogenetically informative with the change observed in more than one taxon and included 9 first-position and 31 third-position transversions, and 34 first-position, 12 second-position and 147 third-position transitions. The 1,140 base-pair sequences had predicted translation products of 379 amino acids. DNA of all taxa terminated with an AGA stop codon.

Base Composition

The base compositions of the three codon positions for all species in this study are similar to those described by Irwin et al. (1991) for the cytochrome *b* gene of 17 ungulate species (Table 4 - 4). Although there is bias in base composition, especially in the silent third-position of codons, the pattern of bias is essentially the same for all species and should not interfere with these comparisons.

Pairwise Comparisons

Pairwise comparison of sequence differences between taxa can provide a simple estimate of evolutionary distance. The sequence divergence among the 11 Caprinae species based on all substitutions ranged from 3.4 % to 14.9 % (Table 4 - 5),

Table 4 - 4. Percentage base composition by species and codon position.

SPECIES	FIRST POSITION				SECOND POSITION				THIRD POSITION			
	A	T	C	G	A	T	C	G	A	T	C	G
MUSKOX	31.6	20.8	26.6	21.1	20.3	41.6	24.5	13.7	44.2	14.2	37.6	3.9
GORAL	30.8	22.1	25.0	22.1	20.0	41.3	24.7	13.9	43.7	17.9	35.3	3.2
SEROW	31.1	22.0	25.2	21.7	19.1	41.4	24.5	15.0	44.7	15.7	35.8	3.8
MT GOAT	28.7	23.1	24.9	23.4	18.0	42.9	24.2	14.9	46.7	16.5	35.2	1.6
SHEEP	31.3	21.1	25.5	22.1	20.3	42.1	23.9	13.7	42.9	18.7	35.3	3.2
BH SHEEP	29.9	22.0	25.3	22.9	18.3	43.6	23.5	14.6	45.3	18.7	33.9	2.1
DL SHEEP	31.9	22.3	23.8	22.0	19.5	41.8	23.8	14.9	44.6	18.3	34.7	2.5
TAKIN	31.1	21.3	25.3	22.4	20.5	42.6	23.2	13.7	44.5	16.6	36.3	2.6
TAHR	32.5	22.4	24.2	20.9	19.7	41.2	24.8	14.3	44.9	15.3	36.2	3.6
GOAT	31.6	20.8	26.1	21.6	20.3	41.8	24.2	13.7	44.2	15.8	36.8	3.2
SAIGA	29.7	21.2	28.5	20.6	20.6	40.3	23.8	15.2	45.3	18.7	33.9	2.2
COW	28.7	22.4	26.1	22.9	20.5	40.5	25.3	13.7	44.5	12.6	39.2	3.7
MEAN	30.8	21.7	25.5	22.0	19.8	41.8	24.2	14.2	44.6	16.5	35.9	3.0
ST. DEV.	1.18	0.70	1.16	0.82	0.84	0.91	0.57	0.58	0.89	1.89	1.46	0.72
BIAS[a]	0.084				0.224				0.407			

[a] Bias in base composition as described by Irwin et al. (1991).

Table 4 - 5. Pairwise sequence comparisons. Percent divergence based on all aubstitutions (above diagonal). transition:transversion ratios (below diagonal).

SPECIES	Muskox	Goral	Serow	MT Goat	Sheep	BH Sheep	DL Sheep	Takin	Tahr	Goat	Saiga	Cow
Muskox	-	11.3	9.7	11.1	12.7	11.0	12.3	11.9	11.1	11.7	14.9	14.1
Goral	8.67	-	9.8	13.1	13.0	11.9	11.5	12.7	10.7	12.0	14.9	16.4
Serow	4.42	4.31	-	12.3	11.3	11.5	11.0	11.9	10.3	9.8	13.3	15.2
MT Goat	4.44	4.53	3.14	-	11.5	10.8	12.1	12.8	12.5	12.8	14.6	15.2
Sheep	6.38	7.42	3.82	3.76	-	7.3	6.6	8.3	11.5	9.8	14.6	16.5
BH Sheep	4.71	5.07	3.20	3.69	15.00	-	3.4	6.3	11.3	10.5	13.2	15.3
DL Sheep	4.88	5.27	3.15	3.35	15.33	3.00	-	5.9	10.7	9.1	13.1	15.1
Takin	4.00	4.88	2.90	3.14	12.75	4.14	11.67	-	11.5	10.6	14.5	16.5
Tahr	5.69	5.43	3.53	4.26	6.33	4.93	5.62	5.14	-	8.0	14.2	14.8
Goat	7.36	6.50	3.73	4.41	6.30	4.69	5.36	5.17	9.17	-	14.6	14.1
Saiga	2.34	2.27	1.61	2.26	2.11	1.69	1.83	2.12	2.12	2.15	-	16.7
Cow	2.18	2.83	2.14	2.23	2.74	2.11	2.37	2.28	2.44	2.83	2.16	-

Table 4 - 6. *Pairwise sequence comparisons. Percent divergence based on transversions; all codon positions (above diagonal), third position of codons only (below diagonal).*

SPECIES	Muskox	Goral	Serow	MT Goat	Sheep	BH Sheep	DL Sheep	Takin	Tahr	Goat	Saiga	Cow
Muskox	-	1.2	2.1	2.4	1.7	2.4	2.1	2.1	1.8	1.5	4.3	4.5
Goral	1.6	-	2.0	2.3	1.5	2.4	1.7	1.9	1.6	1.7	4.5	4.1
Serow	2.4	1.6	-	2.9	2.4	2.9	2.8	2.8	2.0	2.4	5.1	4.7
MT Goat	4.0	4.0	4.8	-	2.3	2.6	2.5	2.8	2.3	2.6	4.4	4.9
Sheep	4.0	3.2	4.0	5.6	-	0.9	0.4	0.7	1.7	1.4	4.7	4.2
BH Sheep	3.6	3.6	4.4	5.2	0.4	-	0.7	1.5	1.9	2.2	4.6	4.6
DL Sheep	5.2	4.4	5.2	6.8	1.2	1.6	-	0.4	1.4	1.2	4.3	4.1
Takin	4.8	4.0	4.8	6.4	0.8	1.2	0.4	-	1.8	1.7	4.4	4.5
Tahr	3.6	2.8	3.6	5.2	3.6	4.0	4.0	3.6	-	1.0	4.7	4.5
Goat	3.6	2.8	3.6	5.2	3.6	4.0	4.0	3.6	1.6	-	4.7	3.9
Saiga	9.2	9.2	10.0	8.4	10.0	9.6	10.4	10.0	8.8	9.6	-	5.3
Cow	10.4	9.6	10.4	9.6	10.4	10.8	10.8	10.4	10.0	9.2	10.8	-

consequently saturation of transition sites should not be a problem in phylogenetic analyses (Kraus and Miyamoto, 1991). Transition:transversion ratios, which provide another measure of divergence, should decrease with increasing distance between taxa as transition sites gradually become saturated (Brown et al., 1982). The highest ratios occurred among the sheep, all closely related species, while the lowest ratios were between the divergent saiga (*Saiga tatarica*) and other Caprinae (Table 4 - 5).

Parsimony Analysis

A single most-parsimonious tree was produced when all 233 phylogenetically informative sites were included in the analysis and cow was designated an outgroup (Fig. 4 - 1). This tree had a length of 613 mutational steps and a consistency index (CI) of 0.47. The g_1 statistic was -0.49, indicating a significant skew to the tree-length distributions, and therefore a strong phylogenetic signal in these data (Hillis and Huelsenbeck, 1992). Because transitions accumulate more rapidly than transversions, especially at third positions of codons (Brown et al., 1982), parsimony may be more accurate if some or all transition sites are given less weight or ignored. Parsimony trees were generated weighting transversions four times as much as transitions (233 sites included), excluding all third-position transitions (86 sites included) and using transversion sites (40 sites included) only. A single most-parsimonious tree resulted from each of these approaches. The tree topology with weighted transversions was identical to the first parsimony tree (Fig. 4 - 1), but the other two trees differed slightly from each other and from the first parsimony tree (Figs. 4 - 2 & 4 - 3). These trees had lengths of 928, 214 and 103, and CI's of 0.52, 0.56 and 0.61, respectively. The g_1 statistics for these trees (-0.59, -0.63 and -0.60) were all significant as well. Among the three topologies, the differences were the placement of the goral (*Nemorhaedus caudatus*) and serow as sister taxa and the placement of the tahr (*Hemitragus jemlahicus*)/goat (*Capra hircus*) clade closer either to the sheep or Rupicaprini.

Distance Analysis

To evaluate the relationships among all taxa from a different perspective, the neighbor-joining method from PHYLIP 3.53 (Felsenstein, 1993) was used. Distances were calculated using the Kimura 2-parameter model with different rates for transitions

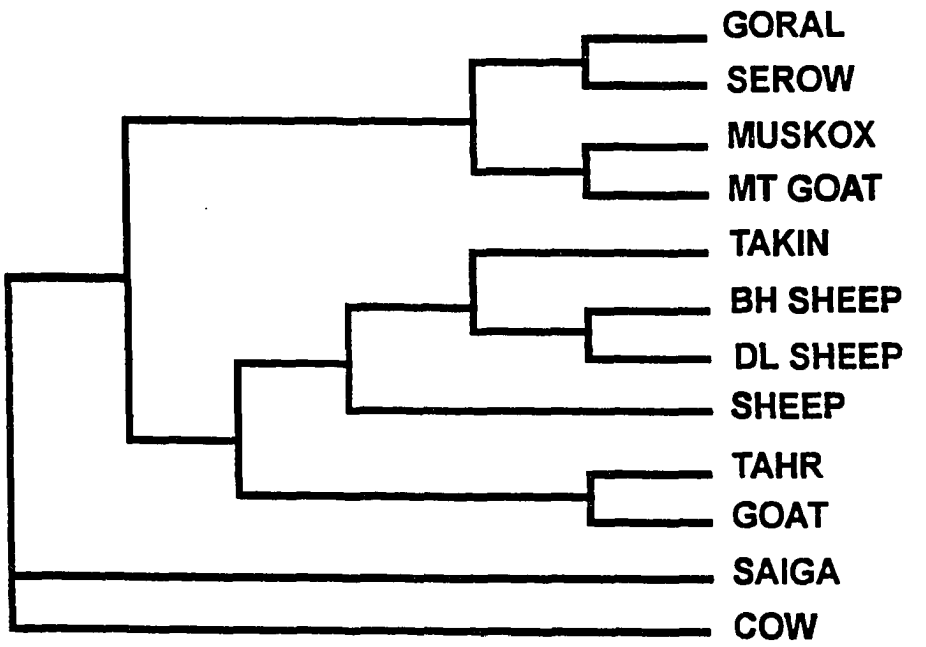

Figure 4 - 1. Parsimony tree based on all informative sites. Length = 613, CI = 0.47, g_1 = -0.49, and log likelihood = -5,202.7. This same topology was obtained with transversion weighted four times as much as transitions. Length = 928, CI = 0.52 and g_1 = -0.59.

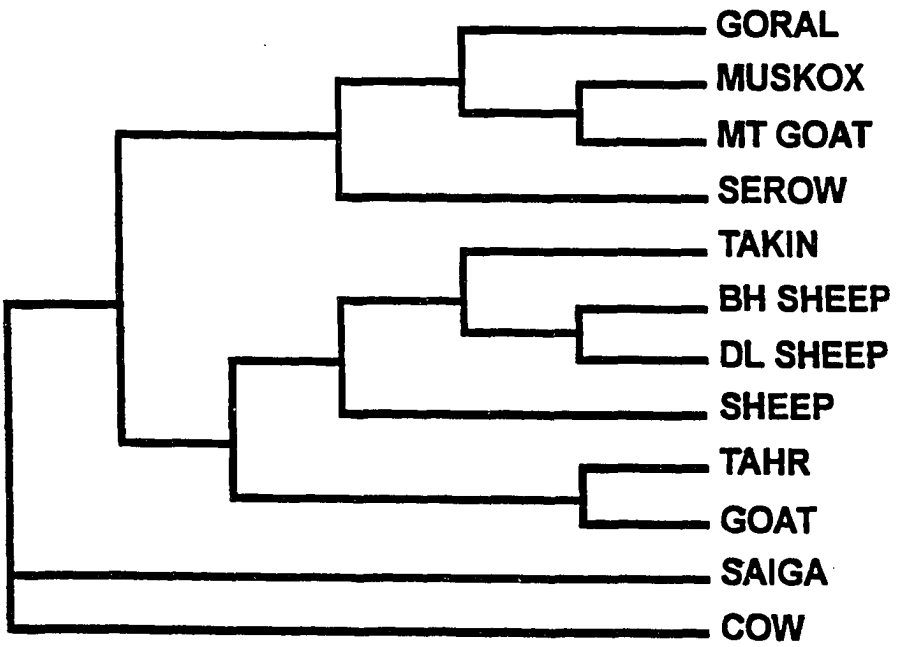

Figure 4 - 2. Parsimony tree excluding all third-position transitions. Length = 214, CI = 0.56; g_1 = -0.63 and log likelihood = -5,208.0.

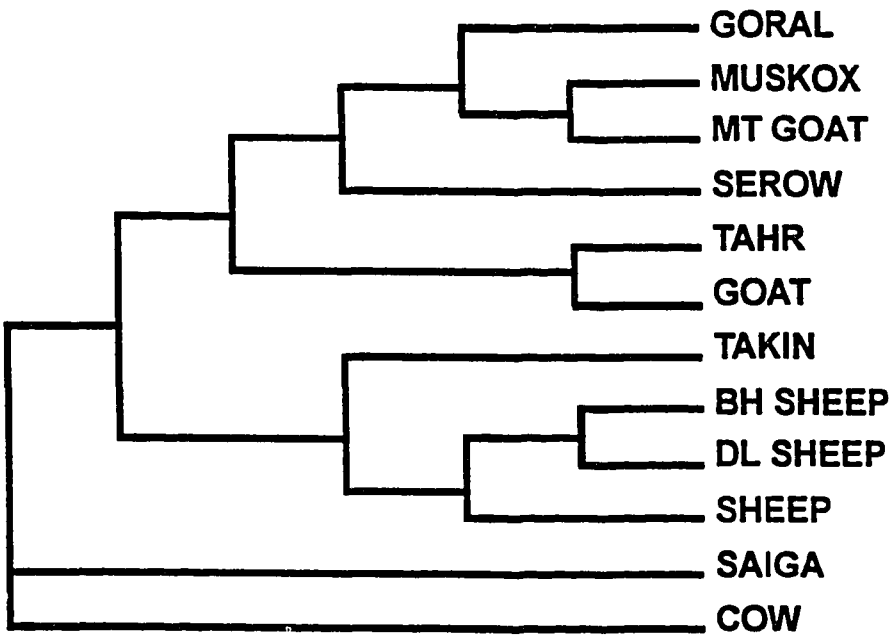

Figure 4 - 3. Parsimony tree including only transversion sites. Length = 103, CI = 0.61, *g*1 = -0.60 and log likelihood = -5,215.8.

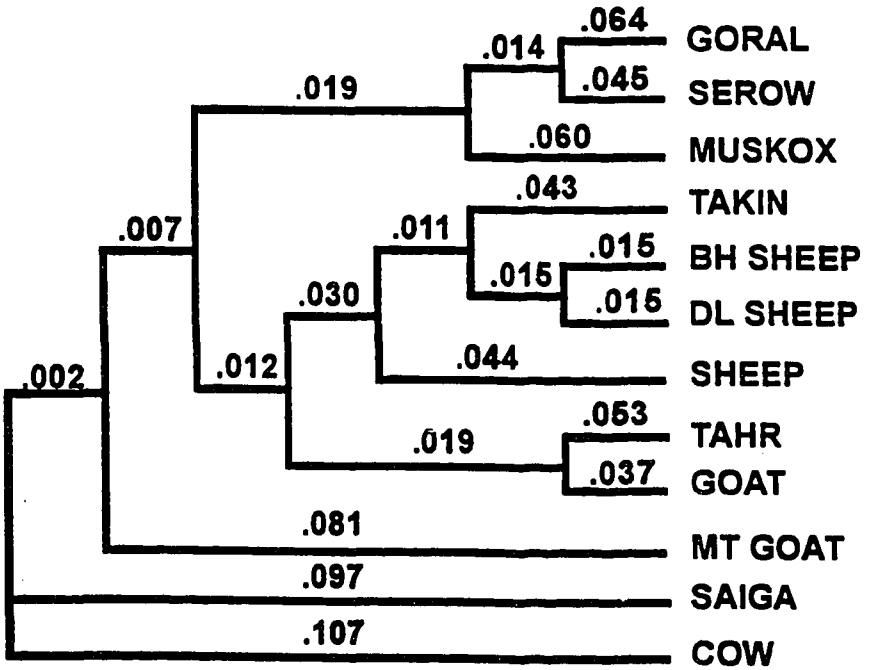

Figure 4 - 4. Neighbor-joining distance tree with individual branch lengths noted. Log likelihood = -5,207.9.

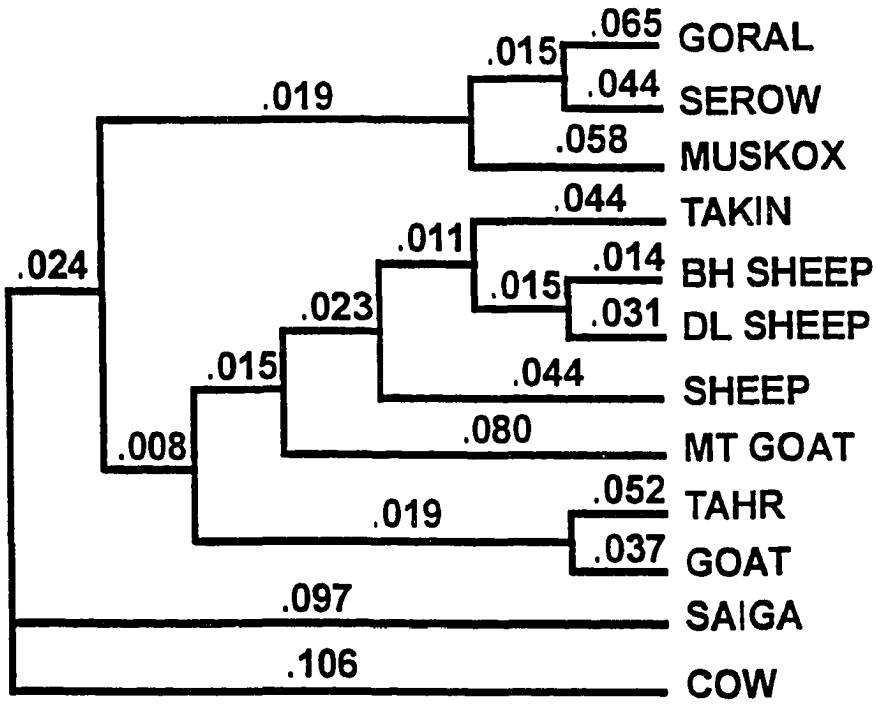

Figure 4 - 5. Maxium likelihood tree (DNAML) with individual branch lengths. Log likelihood = -5,201.7.

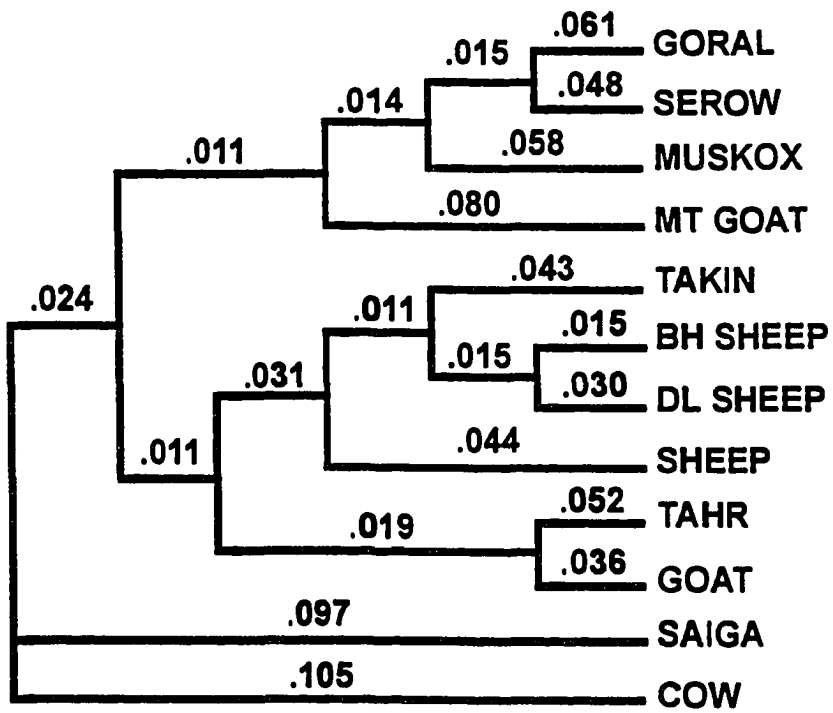

Figure 4 - 6. Maximum likelihood with molecular clock tree (DNAMLK) with individual branch lengths. Log likelihood = -5,203.0.

and transversions. The topology of this distance tree was similar to the first parsimony tree except that mountain goat (*Oreamnos americanus*) was placed outside the Rupicaprini clade (Fig. 4 - 4).

Maximum Likelihood Analysis

A maximum-likelihood (ML) tree was estimated with DNAML from PHYLIP 3.53 (Felsenstein, 1993), using the observed nucleotide frequencies and a transition:transversion ratio of 4.1 which was found to maximize the likelihood. This tree (Fig. 4 - 5) was different from the parsimony and distance trees in that the mountain goat was placed in the Caprini rather than Rupicaprini clade.

A second maximum likelihood tree, using the same parameters as above, was estimated with DNAMLK from PHYLIP 3.53 (Felsenstein, 1993) which is similar to DNAML, but assumes a molecular clock. This tree (Fig. 4 - 6) differed from the DNAML tree in the placement of the mountain goat closer to the Rupicaprini clade.

The log likelihood of different tree topologies can be calculated and compared using DNAML. The three parsimony trees, the distance tree and the two maximum likelihood trees were all compared using this approach. The ML tree (without a molecular clock) had the highest (-5,201.7), and therefore "best" likelihood, but was not significantly better than the other trees. The second "best" tree was the parsimony tree using all informative characters (-5,202.7). The major difference among all trees was the placement of the mountain goat.

DISCUSSION

In this study, parsimony, distance and maximum likelihood methods of analysis all yielded slightly different trees, but there was no significant difference among the log likelihoods of these trees. While the maximum-likelihood tree has the highest likelihood, it cannot be unequivocally selected as the "best" tree. This lack of resolution appears to be partially because of unequal rates of evolution among the taxa and the consequent, unequal branch lengths. In addition, the rapid radiation of the Caprinae has probably resulted in enough homoplasy to reduce the resolving power of this phylogenetic analysis.

Maximum parsimony has commonly been used in phylogenetic analysis (Felsenstein, 1988), but unequal rates of evolution between taxa and the occurrence of

homoplasy, or the independent derivation of the same character, can mask the true phylogeny if parsimony is relied upon to find the shortest tree (Stewart, 1993). High levels of homoplasy essentially will result in a random data set with little or no phylogenetic signal. The strength of the phylogenetic signal can be evaluated with the skewness test statistic, g_1 (Hillis and Huelsenbeck, 1992). Although our parsimony trees all had significant g_1 statistics, the low consistency indices suggest enough homoplasy to reduce the resolving power of parsimony analysis for this data set.

Distance methods use a matrix of pairwise distances between taxa to create a tree with minimum branch lengths (Felsenstein, 1988). Distance methods, particularly the neighbor-joining method (Saitou and Nei, 1987) using the Kimura two-parameter model (DeBry, 1992), can be consistent in finding the correct tree when rates of evolution are equal among branches, but do not perform as well when the rates differ (Hasegawa et al., 1991). The unequal branch lengths of the distance tree (Fig. 4 - 4) indicate that unequal rates of evolution may make distance analysis inappropriate for our data.

The maximum-likelihood phylogenetic method chooses a tree which maximizes the likelihood of the data set fitting a particular model of evolution (Felsenstein, 1988). Maximum likelihood is robust against some violations of the model such as the transition:transversion ratio and unequal rates of evolution among branches (Hasegawa et al., 1991), but the computational time involved makes examining all possible tree topologies difficult. When all branches in a tree are of equal length, maximum-parsimony, neighbor-joining (using the Kimura model) and maximum-likelihood methods all have similar probabilities of estimating the correct tree for four taxa with 1,000 nucleotide sites (Hillis et al., 1994). With unequal branch lengths, however, the probabilities decline dramatically for all but the maximum-likelihood method. In this situation, the efficiency of maximum likelihood declines so that significantly more nucleotide sites are required for a 95% probability of estimating the correct tree (Hillis et al., 1994). Because of unequal branch lengths and the size of our data set, maximum likelihood may not have estimated the best tree.

Despite the inability to define one "best" tree topology, certain groupings remain consistent between all the analyses and can be considered stable clades. These

include the goral, serow, muskox clade, the takin and all sheep clade, and the tahr and goat clade. Saiga and cow are consistently more divergent than the other taxa. Because cow was included as an outgroup and the Saigini is recognized as highly divergent from the other Caprinae, these two species were expected to separate from the remaining taxa.

Mountain goat is the only taxon that does not maintain a stable relationship with any other taxa. The pairwise sequence comparisons (Tables 4 - 5 & 4 - 6) suggest a higher rate of evolution of the mountain goat from other species. Among all four pairwise comparisons, mountain goat consistently lacks a low level of divergence from any other species. Assuming mountain goat must have shared a common ancestor with at least one of these clades, the high level of divergence is best explained by a faster rate of evolution. We propose that because the mountain goat has evolved at a more rapid rate than the other taxa, the present data set is inadequate to place the mountain goat confidently within one of the stable clades. At least one restriction fragment length polymorphism (RFLP) of mtDNA is shared among the mountain goat and sheep, but not muskox (Cronin, 1994). Although this supports the topology of our maximum-likelihood tree with mountain goat closer to the sheep than Rupicaprini, one RFLP does not provide enough information to solidly support a relationship. More species and sites must be included in the analysis to confidently place mountain goat within the Caprinae phylogeny.

The stable clades basically follow the traditional tribal classification of the Caprinae except for the Ovibovini. Our analysis clearly separates the takin and muskox into different clades; the takin always clusters with the sheep in the Caprini whereas the muskox clusters with the Rupicaprini. The observed morphological (i.e. body size and horn shape) and behavioral (i.e. social structure and group defense) similarities between the takin and muskox that caused them to be classified in the same tribe appear to be a result of convergent evolution rather than a shared heritage (Groves and Shields, manuscript).

Chromosomal comparisons have traditionally been used to infer relationships among species. Within the Caprinae, however, karyotypes range from diploid numbers ($2n$) of 60 to 42 (Schaller, 1977) and do not provide a clear pattern of relationships. In

addition, chromosomes may evolve at different rates from morphology and behavior, contributing confusion to proposed phylogenies. These differing rates have been proposed for Siberian snow sheep (*Ovis nivicola),* which is believed to have descended from the North American mountain sheep (*Ovis dalli* and *O. canadensis*). Both species of North American sheep have $2n = 54$ while the snow sheep has $2n = 52$, a more recently evolved karyotype. Snow sheep have retained behavior and morphology that appears ancestral to the North American species (Korobitsyna et al., 1974). A similar pattern has been observed between tahr (*Hemitragus*) and goats (*Capra*). All species of goats have retained the ancestral karyotype of $2n = 60$, while those of tahr have a more evolved karyotype of $2n = 48$ (Bunch and Nadler, 1980). At the same time, goats are regarded as being more evolved morphologically and behaviorally than the tahr species (Geist, 1971). Our analysis consistently clusters the domestic goat (*Capra hircus*) and Himalayan tahr (*Hemitragus jemlahicus*) suggesting a close relationship between the genera despite their divergent patterns of chromosomal, morphological and behavioral evolution.

The placement of the muskox within Rupicaprini and the takin close to the sheep in addition to the pairwise divergences (Tables 4 - 5 & 4 - 6) indicated by our data, support the contemporaneous separation of Rupicaprini and Caprini suggested by Randi et al. (1991). The separation of goats and sheep proposed by Hartl et al. (1990), however, is not clearly supported by our data. Our analysis, except for transversion parsimony (Fig. 4 - 3), places goats slightly closer to sheep suggesting the tribal grouping of Caprini with both goats and sheep is still accurate. However, the clustering of the tahr with the goat indicates more separation between goats and sheep than traditionally thought (Geist, 1987). More species and genera must be analyzed to thoroughly investigate this group.

Within *Ovis*, two distinct groups are generally recognized: the mountain sheep (subgenus *Pachyceros*) which include bighorn, Dall's and snow sheep and the European and Asiatic sheep, which include all the other species in the genus (Table 4 - 1). Two theories have been proposed for the evolution of these two groups. The first is that all sheep are monophyletic and evolved from a common ancestor, the second is that the two groups are polyphyletic and that the $2n = 54$ karyotype observed in both

groups evolved independently from non-random fusions (Nadler et al., 1973). Our data support the polyphyletic origin of sheep with the placement of the takin between the *Pachyceros* sheep and domestic sheep. Under this scenario, ancestral takin would have diverged from ancestral *Pachyceros* after the split from the other sheep and then presumably remained isolated from all sheep long enough to have developed its distinctive characteristics. Analysis of sequences from other sheep and Caprini is necessary to support this hypothesis.

The relationship of gorals and serows has generated debate. Based on examination of museum skins and skulls, Groves and Grubb (1985) suggested combining the two genera (*Nemorhaedus* and *Capricornis*) into one genus, *Nemorhaedus*. Geist (1987) agreed with this reclassification. But, while both gorals and serows are regarded as less evolved species (Schaller, 1977), and have morphological similarities, our sequence data (Tables 4 - 5 & 4 - 6) show more divergence between goral and serow than between tahr and goat, two unquestionably distinct genera. In addition, genetic comparisons support their separation into two genera. Gorals have a $2n = 56$ while serows have $2n = 48$ or 50 (Soma et al., 1987). In conjunction with our sequence data, these karyotypes are different enough to suggest divergent paths of evolution for the two groups. Thus we conclude that the two separate genera should be maintained.

Morphological, behavioral and cytogenetic characteristics appear to evolve at rates independent of one another, and based on our analysis, independent of the rate of evolution of DNA. These different rates presumably are because of the different constraints and selective pressures operating on each of these features. Since the rates also can differ between taxa, each characteristic can be expected to present slightly different phylogenies that appear to contradict each other and the entire phylogenetic picture can become extremely complicated.

Incongruencies between morphological and molecular phylogenies have been recognized and debated upon ever since molecular techniques have been in use (Patterson, 1987). Among some mammalian clades (i.e. primates and bats) morphological and molecular phylogenies appear to contradict each other (Novacek, 1992). In a study of laboratory mice (*Mus musculus*), Fitch and Atchley (1987) found

that trees generated from molecular data agreed with known phylogenies while morphological trees did not. The difficulty of distinguishing between homologous and analogous features can complicate the establishment of an accurate phylogeny based on morphological characteristics (Goodman et al., 1987) as can the occurrence of homoplasies in phylogenies based on DNA sequence data (Patterson et al., 1993).

Traditional phylogenetics have been based on the assumption that characters such as morphology and behavior change slowly over time and that as species evolve, they select environments best suited to these characteristics. We believe these traditional phylogenetic characters are highly plastic and can change rapidly to suit environments that species occupy because they are likely to enhance survival through reduced competition and predation or increased availability. Thus, the evolution of these characteristics is controlled more by environmental pressures than by shared ancestry.

DNA is the fundamental unit upon which these other characteristics are based, thus DNA ultimately should provide the most objective phylogeny. Different regions of DNA, however, both nuclear and mitochondrial, are subjected to different constraints and selective pressures, so an array of DNA should be analyzed for comprehensive phylogenetic reconstructions. In addition to the region of DNA analyzed, the amount of sequence and the method of analysis can affect the resulting phylogeny (Patterson et al., 1993), and these variables must be considered when assessing different phylogenies.

The analysis of sequence data from the cytochrome *b* gene suggests some phylogenetic patterns within the Caprinae that deserve more investigation. These include the separation of the takin and muskox, possible polyphyletic origin of sheep, the clustering of tahr and goat, and the rapid rate of evolution of the mountain goat. Although we are unable to completely resolve Caprinae phylogeny, we have proposed some relationships that differ from phylogenies based on morphological, behavioral and cytogenetic characteristics. Analysis of mtDNA sequence data of the tribe Bovini within the Bovidae also contradicted more traditional phylogenies (Miyamoto et al., 1989). Because of their numbers and diversity, classification of the Bovidae always has been problematical (Simpson, 1945). We believe, however, that DNA sequences provide a

more objective indication of phylogeny than more traditional phylogenetic characteristics that may be either more subjectively measured or plastic in nature. Because of the complexities and rapidity of Caprinae evolution, our study, which investigated one gene of mtDNA, would be complemented by the analysis of more mtDNA genes as well as some nuclear DNA regions.

CHAPTER 5 MUSKOXEN HAVE LITTLE INTRASPECIFIC VARIATION BASED ON CONTROL REGION SEQUENCES

INTRODUCTION

The muskox (*Ovibos moschatus*), a species whose natural range is limited to the Arctic and subarctic, has a documented history of dramatic population fluctuations (Tener, 1965). Additionally, small groups have been translocated both to reestablish populations in historically utilized ranges and to introduce them into previously unused habitats (Klein, 1988). Because of these fluctuations and translocations, and their occupation of patchy, insular habitats, muskoxen have gone through significant genetic bottlenecks both in recent, as well as prehistoric times. The impact of these bottlenecks on genetic diversity of muskoxen is not understood, but an electrophoretic study of allozymes uncovered extremely little genetic variability among Greenlandic and Alaskan muskox populations (Fleischman, 1986).

The ability to directly analyze nucleotide sequences of DNA has proved a valuable tool in studies of molecular evolution. In particular, studies of the evolution of the mitochondrial DNA (mtDNA) molecule have advanced understanding of the phylogenies of many organisms (Brown, 1985; Irwin et al., 1991). Within the mtDNA molecule, comparisons of the rapidly evolving control region have provided a powerful technique to resolve intraspecific phylogenies (Avise et al., 1987; Thomas et al., 1990; Edwards, 1993). With the development of the polymerase chain reaction (PCR) and direct-sequencing techniques (Gyllensten and Erlich, 1988), sampling of many individuals using only small amounts of tissue is now practical, so investigating previously unresolved aspects of intraspecific relationships is now possible.

Historically, muskoxen were classified in two genera and five species until Allen (1913) reduced the confusion by proposing one genus, *Ovibos*, one species, *moschatus*, and three subspecies, *moschatus*, *wardi* and *niphoecus*. Based on the lack of statistically significant differences in morphological measurements, Tener (1965) rejected all subspecies of muskoxen. Currently, based on physical characteristics and

geographical separation, two subspecies, barren-ground muskox (*O. m. moschatus)* and white-faced muskox (*O. m. wardi)* are recognized by most authors (Rowell, 1990).

The natural range of the barren-ground muskox is limited to regions of mainland Canada in the Northwest Territories north of treeline (Fig. 5 - 1). The current range of indigenous populations of white-faced muskoxen extends from about 70° N on the east coast of Greenland, north to the northernmost land on the globe in Greenland (83° 30' N) and westward through most of the arctic islands of Canada except Baffin Island (Fig. 5 - 1). Harington (1961, 1980) speculated that subspeciation occurred during the Wisconsin glaciation (70,000 to 10,000 years BP) when *O. m. wardi* survived in a refugium north of the ice while *O. m. moschatus* retreated south of the ice. As the ice melted, *O. m. wardi* could have dispersed throughout the arctic islands and to Greenland while *O. m. moschatus* moved north with the retreating ice edge to its present range.

Muskoxen disappeared from Alaska sometime during the mid-1800's, possibly due to a combination of environmental factors and hunting pressure (Hone, 1934; Burch, 1977). In 1930, 34 muskoxen were translocated from east Greenland to Alaska to help ensure the survival of the species (Bell, 1931). These animals were ultimately released into the wild on Nunivak Island (Fig. 5 - 1). Since 1967, several groups of muskoxen have been translocated from Nunivak to historical ranges in northwest and northeast Alaska (Klein, 1988). The muskoxen in northeast Alaska have been dispersing east into the Yukon Territory, Canada (P. Reynolds, USFWS, pers. comm.).

In recent decades, populations of barren-ground muskoxen have been increasing and, as they recolonize historic ranges, extending their distribution westward toward the Mackenzie River (Case et al., 1989). The Alaskan animals (white- faced muskoxen) which have moved into the Yukon, are extending their distribution eastward toward the Mackenzie River. If these range expansions continue, the two subspecies have the potential to eventually meet and interbreed. Because of the reintroduction of muskoxen to Alaska, this hybridization of the subspecies would be the result of human interference and of uncertain appropriateness. In order to evaluate the desirability

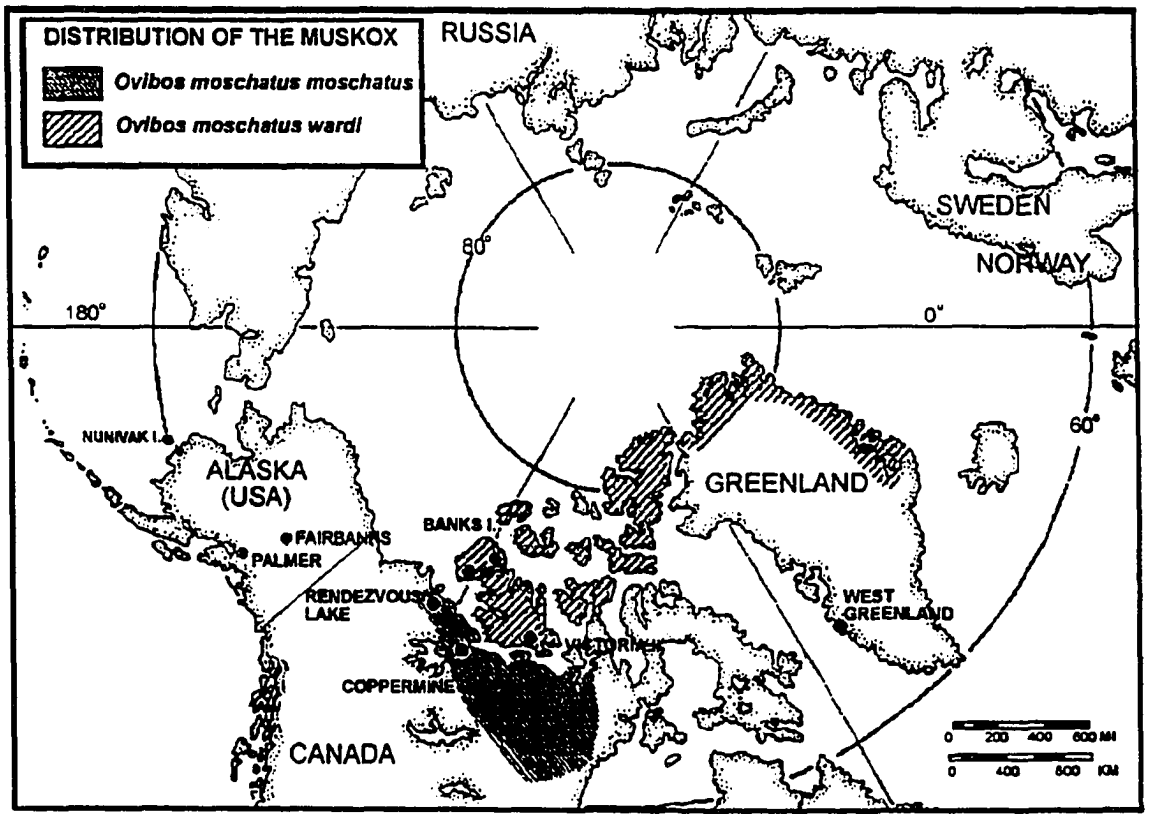

Figure 5 - 1. Natural distribution of muskoxen (shaded areas). Dots (●) indicate locations of populations sampled. Sampled populations in Alaska and Greenland are from stock translocated from east Greenland.

of intermixing of the subspecies, the genetic variability between and within them must be established to determine if the two subspecies are distinct from each other.

In this study, I sequenced portions of the control region of mtDNA from muskoxen from both subspecies, and from some indigenous and some introduced populations to attempt to answer the following questions:

1) Can the two muskox subspecies be distinguished from each other based on control region variation? 2) Should there be concern about the introduced Alaskan muskoxen interbreeding with the indigenous muskoxen of the Canadian mainland and the possible loss of naturally distinct subspecies? 3) Can control region variation be used to establish relationships among muskox populations throughout their range?

METHODS

MtDNA sequences for this study were analyzed from 37 muskoxen from nine different locations (Table 5 - 1, Fig. 5 - 1). The samples included 10 individuals of *O. m. moschatus*, 26 of *O. m. wardi* and one of uncertain ancestry. Except for the uncertain animal, all the Alaskan samples are from muskoxen descended from 34 individuals translocated from Greenland in 1930 and ultimately placed on Nunivak Island. The Palmer muskox herd was started in 1964-5 with 33 animals captured on Nunivak Island, and the Fairbanks herd began in 1979 with 16 animals captured on Nunivak. While the Nunivak, Fairbanks and Palmer herds are now isolated from each other, and referred to as separate populations, their close relationship must be recognized.

DNA was extracted from 300 μl aliquots of whole blood by differentially lysing red and then white blood cells, precipitating residual proteins with high salt and then precipitating the DNA with isopropanol using protocols adapted from Miller et al. (1988) and Winberg (1991). DNA was extracted from 20 μg sections of frozen muscle, heart, placenta and dried skin using standard lysis and digestion protocols (Maniatis et al., 1982) followed by salt and isopropanol precipitations.

Table 5 -1. Populations of muskoxen sampled. Subspecies, location[a], type, sample size (N), genotype and number of individuals (#) with that genotype in population, and source of tissues analyzed.

Subpecies	Location	Tissue Type	N	Genotype (#)	Source
O. m. moschatus	Coppermine, NWT	Blood	5	1 (5)	Anne Gunn, Renewable Resources, Government Northwest Territories(GNWT)
"	Rendezvous Lake, NWT	Muscle	5	1 (2) 2 (3)	Renewable Resources, GNWT
O. m. wardi	Banks Island, NWT	Blood, heart	9	1 (3) 3 (6)	Renewable Resources, GNWT
"	Victoria Island, NWT	Blood	4	4 (4)	Anne Gunn, Renewable Resources, GNWT
"	Nunivak Island, AK	Muscle	1	5 (1)	Hunter killed
"	Fairbanks, AK	Blood, skin	5	5 (5)	Large Animal Research Station, University of Alaska Fairbanks
"	Palmer, AK	Blood, placenta	5	6 (5)	Musk Ox Development Corporation
"	West Greenland	Muscle	2	7 (2)	Greenland Environmental Research Institute, Copenhagen
?[b]	Palmer, AK	Blood	1	8 (1)	Musk Ox Development Corporation

a For muskox distribution and location, see Fig. 5 - 1.

b This individual was born at the Cheyenne Mt. Zoo, Colorado Springs, CO. Her ancestry is uncertain, but she is probably is a mixture of *O. m. moschatus* and *O. m. wardi.*

Portions of the mitochondrial control region were amplified from all samples via PCR using one pair of primers. The primers were provided by Curtis Strobeck, University of Alberta and were designed based on the known bovine mtDNA sequence (Anderson et al., 1982) to lie in the conserved regions of 12S rRNA (5' GGAAGGCTGGGACCAAACCT 3') and proline tRNA (5' TAATATACTGGTCTTGTAAACC 3') and outside the more variable control region. Most amplifications were 50 µl asymmetric reactions with primer ratios between 1:10 and 1:100, which generated single-stranded DNA for sequencing (Gyllensten and Erlich, 1988). Some samples required a double-stranded amplification with primers in equal concentrations followed by a single-stranded amplification with only one primer (Kocher et al., 1989). Amplified DNA was visualized by ethidium-bromide staining of agarose gels. Amplifications in the predicted size range were purified by three centrifugal filtrations with 350 µl of water in Ultrafree MC 30,000 NMWL (Millipore) tubes. DNA was resuspended in 25 µl sterile water and sequenced using the dideoxy sequencing methods of Sanger et al. (1977). Specifically, 7 µl of the PCR product, the limiting primer in the PCR reaction and Sequenase Version 2.0 DNA Sequencing kits (U.S. Biochemical) were used, following protocols described with the kit. Amplification and sequencing reactions for all individuals were conducted using each primer as the limiting one, so sequences were generated for both ends of the control region.

Sequences were read with the help of DNA Parrot DP 100-PC^{TM} (Clontech) and aligned using Align Plus Version 2 (Scientific and Educational Software). Pairwise comparisons were made using MEGA (Kumar et al., 1993). Phylogenetic analyses were conducted using the exhaustive search option of PAUP Version 3.1 (Swofford, 1993) and the DNADIST, NEIGHBOR, DNAML, SEQBOOT, DNAPARS, and CONSENSUS programs within PHYLIP Version 3.5 (Felsenstein, 1993).

RESULTS

Two segments of sequence were generated for all individuals for a total of 860 base pairs (bp). Because of the placement of the primers outside the control region, 163 bp of these sequences were outside the control region and there was no variation among them, so only the 697 bp of the control region were analyzed. Thus, the

sequences analyzed included 272 bp from the left, or 5'-end of the control region and 425 bp from the right, or 3'-end.

Among the 697 bp of all individuals sampled, 10 changes were observed; each change was observed in more than one individual, so all were phylogenetically informative for analysis. These changes included five transitions, one transversion and four insertion-deletion events of a single base pair each (gaps). Eight distinct sequence patterns, or genotypes, were observed among the 37 individuals. These genotypes tended to be restricted to separate populations, although the Rendezvous Lake and Banks Island populations each had two genotypes (Table 5 - 1). Genotype 1 was observed in three populations, and genotype 5 was observed in two populations (Fig. 5 - 2).

Position	1	1	2	4	4	4	4	5	5	5		
	7	8	3	1	1	6	9	2	5	5	I[a]	P[b]
Genotype	3	2	9	1	6	8	7	9	4	7		
1	C	T	-	A	A	C	-	T	C	T	10	3
2	·	·	-	·	·	·	C	·	·	·	3	1
3	·	·	-	·	·	·	-	C	·	·	6	1
4	T	·	-	·	C	·	C	C	·	·	4	1
5	T	·	G	·	·	·	-	·	·	·	6	2
6	·	·	G	·	·	·	-	·	·	·	5	1
7	T	C	G	-	·	T	-	·	T	-	2	1
8	T	·	G	·	·	·	C	·	·	·	1	1

a Number of individuals with genotype.
b Number of populations in which genotype was observed.

Figure 5 - 2. Variable sites in the control region of eight types of muskox mtDNA from 37 individuals. Numbering is from the first position of the control region of bovine mtDNA (Anderson et al., 1982) and based on the 697 base pairs of muskox control region which were sequenced. Dots (.) indicate identity with genotype 1. Dashes (-) indicate deletions relative to other genotypes.

Differences among the genotypes were small. Some differed at only one base pair. Pairwise differences between the genotypes, based on all changes, ranged from 0.14 % to 1.29% (Table 5 - 2). With gaps excluded, the pairwise differences ranged from 0.0 % to 0.72% (Table 5 - 3).

With so little difference among the genotypes, phylogenetic analysis was not effective in resolving relationships among them. The distance and maximum-likelihood methods in PHYLIP (Felsenstein, 1993) treat gaps as unknown sequence and ignore them, so analysis with these methods relied on the differences resulting from the six substitutions. Trees generated with these methods had most branch lengths that were not significantly different from 0.0, and therefore were meaningless.

Maximum parsimony methods can use information from gaps by coding them as a fifth character state. However, parsimony methods begin to fail when the number of phylogenetically informative characters approaches, or is less than, the number of genotypes being compared (Stewart, 1993). In this situation, many most parsimonious trees will be found. With gaps included, this data set has 10 phylogenetically informative characters, but among the eight genotypes, only two differ from each other by nine characters (Fig. 5 - 2). With the eight genotypes and domestic cow (*Bos taurus)* sequence (Anderson et al., 1982) included as an outgroup, maximum parsimony found 39 most parsimonious trees. The g_1 statistic for the tree-length distribution of all 135,135 possible trees was -0.191 indicating no significant skew to the distribution and a lack of phylogenetic signal (Hillis and Huelsenbeck, 1992). The lack of phylogenetic information was emphasized by bootstrapping the data set 1000 times with SEQBOOT, running DNAPARS on the 1000 data sets and computing the consensus tree with CONSENSUS. This consensus tree (Fig. 5 - 3) only shows strong support for separating cow from all the muskoxen and genotype 7 from the other seven muskox genotypes.

DISCUSSION

Sequences from the control region of muskoxen revealed little variability. The DNA sequenced included most of region I, the first 400 bp of the control region, which has been established as the most hypervariable region of mtDNA containing significant intraspecific variation in humans, rodents, cattle and birds (Vigilant et al., 1989; Thomas et al., 1990; Edwards, 1993). The variability did follow the typical pattern for

Table 5 - 2. Pairwise comparisons between 697 base pairs of 8 genotypes of muskox control region. Percentages of all differences (substitutions and insertions/deletions) and insertions/deletions, above and below diagonal, respectively.

Type	1	2	3	4	5	6	7	8
1	-	0.0014	0.0014	0.0057	0.0029	0.0014	0.0100	0.0043
2	0.0014	-	0.0014	0.0043	0.0043	0.0029	0.0115	0.0029
3	0.0	0.0014	-	0.0043	0.0043	0.0029	0.0115	0.0057
4	0.0014	0.0	0.0014	-	0.0057	0.0072	0.0129	0.0043
5	0.0014	0.0029	0.0014	0.0029	-	0.0014	0.0072	0.0014
6	0.0014	0.0029	0.0014	0.0029	0.0	-	0.0086	0.0029
7	0.0043	0.0057	0.0043	0.0057	0.0029	0.0029	-	0.0086
8	0.0029	0.0014	0.0029	0.0014	0.0014	0.0014	0.0043	-

Table 5 - 3. Pairwise comparisons of substitutions between 697 base pairs of 8 genotypes of muskox control region. Percentages of transitions and transversions, above and below diagonal, respectively.

Type	1	2	3	4	5	6	7	8
1	-	0.0	0.0014	0.0029	0.0014	0.0	0.0057	0.0014
2	0.0	-	0.0014	0.0029	0.0014	0.0	0.0057	0.0014
3	0.0	0	-	0.0014	0.0029	0.0014	0.0072	0.0029
4	0.0014	0.0014	0.0014	-	0.0014	0.0029	0.0057	0.0014
5	0.0	0.0	0.0	0.0014	-	0.0014	0.0043	0.0
6	0.0	0.0	0.0	0.0014	0.0	-	0.0057	0.0014
7	0.0	0.0	0.0	0.0014	0.0	0.0	-	0.0043
8	0.0	0.0	0.0	0.0014	0.0	0.0	0.0	-

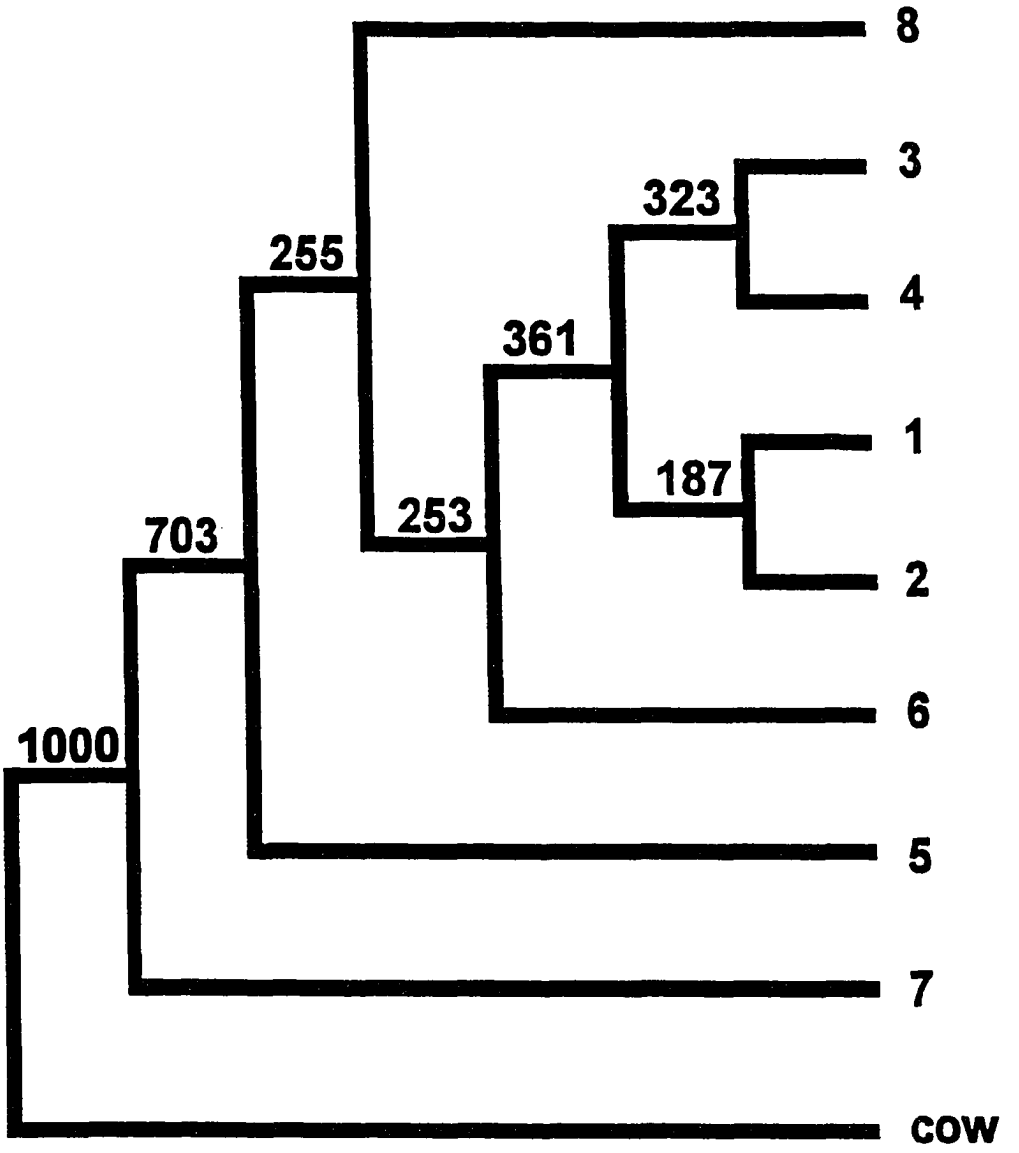

Figure 5 - 3. Consensus tree of 1000 maximum parsimony trees generated from bootstrapped data sets. Numbers 1 - 8 represent eight distinct genotypes of control region sequence of muskox mtDNA. Cow was included as an outgroup. Numbers on branches indicate number of times grouping of genotypes to the right occurred out of 1000 trees.

control region substitutions with transitions being more frequent than transversions (Kocher and Wilson, 1991) and with some gaps which are more common in the control region than other areas of mtDNA (Avise et al., 1987).

Subspecies

Because of the low levels of variability among all the populations sampled, distinguishing the two muskox subspecies based on control region variation is not possible. The division between the subspecies has been defined as the separation between the Canadian mainland and the arctic islands (Harington, 1961), yet genotype 1 occurred in populations on the mainland (Coppermine and Rendezvous Lake) as well as on Banks Island (Table 5 - 1). The difference between genotype 1 and genotype 3, the other genotype found on Banks Island, is only one transition (Table 5 - 3), so there is a possibility this is a homoplasy, or character that evolved independently in both populations rather than a synapomorphy, or shared derived character. The differences between genotypes 1 and 2, the two genotypes for the barren-ground subspecies, and genotypes 4, 5 and 6, supposed white-faced subspecies genotypes, are so small (Table 5 - 2) there is little support from this data set for separating muskoxen into two subspecies.

Support for two subspecies of muskoxen has relied on morphological differences, mostly size and coloration, and an apparent geographic barrier, of water or ice, between these two groups (Harington, 1961; Rowell, 1990). Nevertheless, Tener (1965), who found no statistically significant differences in morphometric measurements between the subspecies, has suggested variations in size of muskoxen may be the result of environmental differences and qualities of diet available to them across their range. Tener (1965) also predicted that the relative uniformity of the arctic environment and lack of effective geographic barriers to movement would reduce the opportunity for muskoxen to differentiate and contribute to genetic homogeneity of the species.

Interbreeding

Based on the low level of variability in control region sequences between muskoxen in Alaska and on the Canadian mainland, there appears to be little reason to be concerned about the mixing and hybridization of these populations and potential

loss of genetically distinct subspecies. The presence of genotype 1 on both the mainland and Banks Island indicates mixing of the two populations already may have occurred in a north-south direction.

The muskoxen sampled from Alaska and the Canadian mainland do have different genotypes (Table 5 - 1), but the difference between them is small. The differences between these Alaskan and Canadian animals are smaller than those between the Alaskan and Greenland animals (Fig. 5 - 2) which are supposed to be the same subspecies. Thus, based on this control region analysis, there appears to be no reason to try to prevent the mixing of the Alaskan and Canadian mainland muskoxen.

Interpopulation relationships

Variation in the control region does not provide enough information to establish relationships among muskox populations throughout their range. Although most of the populations have distinct control region genotypes, the differences among them are too small to provide significant phylogenetic information. Because of known histories of some of the populations, however, potential patterns of relationships can be discerned.

Eleven individuals descended from the stock introduced into Alaska in 1934 were sampled. These animals had two genotypes, one found on Nunivak Island and in the captive herd at Fairbanks, the other found in the herd at Palmer (Table 5 - 1). The difference between these genotypes was only one transition (Fig 2). With just one individual sampled from Nunivak Island, the source population for both the Fairbanks and Palmer herds, the occurrence of both genotypes 5 and 6 on Nunivak cannot be verified.

The animals sampled from the Palmer herd were one to three generations removed from Nunivak-born animals. All were descended from different females captured on Nunivak in 1964 and 1965, whose maternal relationships to each other cannot be known. Because of the maternal inheritance of mtDNA with no recombination (Brown, 1985), genotype 6 must have been present in all the females captured on Nunivak, and could not have derived and become fixed in the Palmer population after removal from Nunivak.

Among the Fairbanks animals sampled, two were a mother-daughter pair and would be expected to have the same genotype. Four of the five individuals sampled

from Fairbanks were the offspring of wild females captured on Nunivak, so their exact maternal relationships are not known. The animal sampled directly from Nunivak had the same genotype (5) as the Fairbanks animals.

This evidence indicates genotypes 5 and 6 both originated on Nunivak Island. That the Fairbanks and Palmer herds have different genotypes can be explained by capture methods and muskox social ecology. During a capture, efforts are usually made to collect several animals from one group. Muskoxen are a polygynous species and the females in a group tend to be maternally related (Smith, 1976). Thus, female muskoxen captured from a group would be expected to share the same mtDNA genotype. Apparently the captures for the two herds included groups with different mtDNA genotypes, but because of the overall low variability of muskoxen, each capture collected some females with identical genotypes. More extensive sampling of the Palmer and Fairbanks herd is necessary to determine if all the animals in each herd have the same genotype.

Whether both genotypes 5 and 6 were present in the animals originally brought from Greenland, or whether one has evolved since 1930 cannot be known at this time. The animals translocated to Alaska were captured from areas around Clavering Island and Hold with Hope in east Greenland (Henrichsen, 1982). Sampling of animals from those areas would provide an indication of the occurrence of either genotype 5 or 6 in the modern indigenous populations and aid in reconstruction of the mtDNA history of the Alaskan animals.

The two muskoxen from Greenland (genotype 7) were the most different from all the other populations (Table 5 - 2, Fig. 5 - 2). These animals were from a population of 27 muskoxen translocated from east Greenland to west Greenland in 1962-65 (Vibe, 1967). Because the present Alaskan muskoxen also are descended from east Greenland stock, the two groups might be expected to have more similar genotypes. The animals for the west Greenland transplant were captured near Scoresbysund (Andersen, 1966), south of the capture locations for the Alaskan transplant. Based on differing frequencies of dental anomalies that may serve as genetic markers, Henrichsen (1982) has suggested that the Scoresbysund muskoxen and those further north belong to separate populations. A geographical barrier which limits movements

between the areas may have isolated the southern population long enough for it to become genetically distinct. Thus, if the transplanted Alaskan and west Greenlandic muskoxen are derived from different populations, they can be expected to have different mtDNA genotypes.

The individual with genotype 8 presents an interesting case. She was born in captivity in a zoo. On the paternal side, she is two generations removed from the wild, her paternal grandfather was from Banks Island and her paternal grandmother from Ellesmere Island, both supposed white-faced types. Her maternal lineage has been lost in a series of transfers between zoos, but apparently extends back through at least four generations of breeding in captivity. The sources of wild caught muskoxen for zoos have not always been documented and barren-ground and white-faced muskox types in zoos have sometimes been interbred (Holst, 1990). This animal has the physical appearance associated with the barren-ground type; she is large (318 kg) and more darkly-colored than the white-faced type, so appears to have some barren-ground background on her maternal side. Her genotype (Fig. 5 - 2), especially at position 239, suggests more similarities with the Alaskan and Greenlandic animals than the mainland Canadian animals. I suggest her maternal lineage is comprised of a mixture of barren-ground and white-faced individuals in which the females tended to be white-faced and contributed mtDNA while the males tended to be barren-ground and contributed physical and other non-mtDNA characteristics.

Lack of variation - bottlenecks

Muskoxen appear to be somewhat unusual in their lack of genetic variability. An electrophoretic allozyme comparison of 28 loci from 87 Alaskan and 39 east Greenlandic muskoxen revealed only one polymorphism which was present in both populations and could not differentiate between them (Fleischman, 1986). The Greenlandic animals were from a population different from the source population of Alaskan animals (Henrichsen, 1982; Fleischman, 1986). These levels of allozyme polymorphisms and heterozygosity were low compared to other ungulates, and to mammals in general (Baccus et al., 1983; Nevo et al., 1984). A comparison of 1,044

Table 5 - 4. Control region variation within different species.

Species	# Individuals	# BP Sequenced	# Variable Sites	% Variable Sites	# Genotypes	Reference
Humans (*Homo sapiens*)	117	400	79	20	88	Di Rienzo & Wilson, 1991
Humans (*Homo sapiens*)	63	360	26	7.2	28	Ward et al., 1991
Chimpanzee (*Pan troglodytes*)	3	1135	106	9.3	3	Kocher & Wilson, 1991
Kangaroo rat (*Dipodomys panamintinus*)	106	135	19	14	23	Thomas et al., 1990
Mice (*Mus domesticus*)	216	1000[a]	80	8	56	Prager et al., 1993
Cattle (*Bos taurus & indicus*)	26	910	63	7.0	24	Loftus et al., 1994
Grey-crowned babbler (*Pomatostomus temporalis*)	44	400	21	5.3	22	Edwards, 1993
Brown bear (*Ursus arctos*)	3	257	5	1.9	2	Shields & Kocher, 1991
Polar bear (*Ursus maritimus*)	8	257	4	1.6	2	Shields & Kocher, 1991
Muskox (*Ovibos moschatus*)	37	697	10	1.4	8	this study

[a] Includes flanking tRNAs

bp of the cytochrome *b* gene, a more conservative area of mtDNA than the control region, of 38 muskoxen from both barren-ground and white-faced stock revealed variation at five sites, but only one site was variable in more than one individual (Groves and Shields, submitted manuscript). This is less than intraspecific variation reported for the cytochrome *b* gene of some other mammalian species (Shields and Kocher, 1991; Smith and Patton, 1991; Mouchaty et al., 1995).

The control region of muskox mtDNA also has lower variability than has been reported for other species. Although the sample size for each population in this study was small, the populations themselves were diverse and should provide a relatively accurate overview of variability within muskoxen. The variability of sequences in the control region of some other species, based on the percentage of variable sites, ranges from 20% to 1.6% whereas that of muskoxen was 1.4% (Table 5 - 4). The other low values of 1.6 and 1.9% were for small sample sizes of bears and encompassed a conserved block within the control region (Shields and Kocher, 1991); this study encompassed a wide variety of muskox populations and included the most variable segments of the control region (Vigilant et al., 1989).

Low levels of genetic diversity often have been attributed to a species or population going through a bottleneck (Nei et al., 1975). Northern elephant seals (*Mirounga angustirostris*), the entire species of which survived a severe bottleneck in the late 1800's, were found to be monomorphic at 24 loci (Bonnell and Selander, 1974). Cheetahs (*Acinonyx jubatus*) also have low levels of variability which have been postulated to be the result of a bottleneck followed by inbreeding (O'Brien et al., 1983).

The known history of muskoxen indicates bottlenecks may explain the low levels of variability within the species. Within recorded history, muskox populations, and their distributions have fluctuated dramatically (Tener, 1965). A dramatic decline of muskox numbers in the late 1800's led to concern the species might be in danger of extinction. In an effort to save the species, the Canadian government granted the muskox complete protection from hunting in 1917 (Hone, 1934). Extreme declines in muskox numbers have actually resulted in local extirpations of populations in Alaska, some Canadian islands and northwest Greenland (Hone, 1934; Thing et al., 1984; Barr, 1991).

Muskoxen in Canada began to recover in some areas in the early 1900's, although as recently as the 1960's numbers were still low (Tener, 1965). Subsequently, many populations increased dramatically, particularly those on Banks and Victoria islands, where almost two thirds of the > 100,000 Canadian muskoxen now occur (Gunn, 1990; Ferguson and Gauthier, 1992). Because of the remoteness of some of the arctic islands which muskoxen inhabit in Canada, the status of these populations is not known (Barr, 1991; Ferguson and Gauthier, 1992). Indigenous populations of muskoxen in Greenland have continued to fluctuate with decreases observed in populations in northeast Greenland in the last decade. Current estimates of indigenous Greenland populations are between 9,500 and 12,500 animals although little is known about the status of remote populations (Boertmann et al., 1992).

Archaeological evidence suggests previous fluctuations in muskox numbers extending back to prehistoric times (Harington, 1961; Barr, 1991). These fluctuations probably have been driven by unfavorable climatic conditions as well as human hunting pressure in more recent centuries (Burch, 1977; Thomas et al., 1981). Thomas et al. (1981) have proposed that some refugia exist in the Canadian Arctic Archipelago where muskoxen continue to survive while populations elsewhere die-off. When conditions improve, muskoxen can emigrate from the refugia to recolonize old ranges. Long distance movements (170 - 340 km) of muskoxen, especially those occupying new ranges, have been documented (Grauvogel, 1984; Lundh, 1984; Uspenski, 1984). The arctic islands are generally less than 50 km apart, so muskoxen should be able to cross between the islands on the ice to recolonize them.

The lack of variability among the white-faced muskoxen can be explained by the existence of high arctic refugia and a history of population fluctuations with local extirpations and recolonizations from closely related founding stock. However, if, as Harington (1961) proposed, the white-faced and barren-ground muskox stocks have been separate since the Wisconsin glaciation, these refugia did not lead to differentiation of mtDNA between the two stocks. The close similarity of the two stocks may be attributed to two factors. Prior to their separation by the ice sheets, all muskoxen may have belonged to a common stock that also experienced bottlenecks which reduced variability. Since the retreat of the ice, muskoxen may have crossed

between the Canadian mainland and islands and interbred reducing any existing variability between them. Such crossings have never been documented, but the distances involved do not preclude this possibility (Harington, 1961).

Loss of genetic variability can be minimized following a bottleneck if the surviving founding group is large enough or if the population increases rapidly immediately after the bottleneck (Nei et al., 1975). The sizes of founding populations of indigenous populations are not known, while those of translocated populations have tended to be small, about 30 animals (Klein, 1988). High rates of increase have been documented in some muskox populations (Gunn, 1990), but these usually have occurred after periods of little increase (Klein, 1988). These factors plus the repeated bottlenecks which muskoxen have experienced throughout their history appear to have reduced variability within the species. Avise et al. (1987) predicted a species whose numbers and range expanded dramatically from a single refugium would demonstrate limited mtDNA phylogeographic differentiation. This prediction holds true for muskoxen.

Presently, muskoxen are experiencing a period of success, with high numbers throughout most of their range. The lack of genetic diversity in the species does not appear to be detrimental to its survival at this time. However, the lack of diversity can reduce the genetic plasticity of a species making it more vulnerable to any changes in the environment and ultimately increasing the likelihood of extinction (O'Brien et al., 1983). With predictions of global change and increasing human activity in the Arctic on a scale greater than has occurred in the past, muskoxen may become a vulnerable species unless refugia remain available to them.

CHAPTER 6 THE CASE FOR CONVERGENCE: THE TAKIN AND MUSKOX

INTRODUCTION

The takin (*Budorcas taxicolor*) and muskox (*Ovibos moschatus*) are both large-bodied species within the subfamily Caprinae. The evolutionary history of both species has puzzled many. The muskox was designated as a species within the genus *Bos* (Zimmerman, 1780) until after the genus *Ovibos,* which recognized similarities to both sheep and cattle, was proposed (Blainville, 1816). The generic name for the takin, *Budorcas*, stemming from the Greek words for cow and gazelle, suggests similarities with those animals (Hodgson, 1850). Similarities between the takin and muskox were recognized as early as 1850 when the takin was first described (Hodgson, 1850). A subfamily, Ovibovinae, consisting of only the takin and muskox was proposed in 1898 (Matschie, 1898). More recently Simpson (1945) placed the two species within the Ovibovini, one of four tribes in the Caprinae.

The present classification of the takin and muskox as sister taxa in the Ovibovini within the Caprinae is based largely on morphological, paleontological and cytogenetic comparisons, characteristics traditionally used in phylogenetic reconstructions. My research has focused on two additional characteristics, ecology and mitochondrial DNA, in an attempt to better understand both of these species and how they might be related. I spent a total of six months in 1988 and 1990 studying takin ecology in the Qinling Mountains, Shaanxi Province, China collecting data on group size, habitat use and dietary selection. In 1989 and 1990, I spent a total of 2 1/2 months observing muskox group dynamics on Banks Island, Northwest Territories, Canada. Subsequent to the field studies, I sequenced the cytochrome *b* gene of mitochondrial DNA (mtDNA) from takins, muskoxen and other Caprinae species to evaluate the relationship of the species on a genetic level.

MORPHOLOGY

Early classifications were based on morphological comparisons. Hodsgon (1850) reported that takin ears, tails and muzzles resembled those of the muskox and that the horns of the two species possessed similarities. In addition to the above characteristics, Matschie (1898) reported the forms of the metacarpus, skull, limbs and dew-claws supported a close relationship of the two species. Lönnberg (1900b) described muskox anatomy in detail and believed the resemblance between the species was superficial, but he only had access to a damaged takin skull on which to base this comparison. However, Lander (1919) who was able to examine a dead captive takin, believed anatomical similarities supported the relationship of the species. The above authors all agreed to affinities of the takin and muskox with other Caprinae, but Allen (1913) thought the muskox should be placed in the Bovinae close to the genus *Bison*. Despite some controversy about their morphological similarities, close relationship between the takin and muskox has been generally accepted (Simpson, 1945; Schaller, 1977; Neas and Hoffmann, 1987).

PALEONTOLOGY

Paleontological records often provide support for evolutionary relationships. The fossil record indicates a muskox-type genus, *Boopsis*, appeared in Asia in the Lower Pliocene (Crégut-Bonnoure, 1984). Primitive *Ovibos* fossils from the Pleistocene have been found across Europe, Asia and North America (McDonald and Davis, 1989). The fossil record of the takin is more sparse than that of the muskox, probably due to the mountainous environment in which they evolved (Simpson, 1945). Early takin fossils, *B. teilhardi*, date from the Upper Pliocene in China (Neas and Hoffmann, 1987). No fossil form ancestral to both the takin and muskox has been identified, but Harington (1989) proposed *Budorcas* to be related to the primitive muskox genera *Boopsis* and *Soergalia*.

CYTOGENETICS

Comparative cytogenetic evidence is often used to support phylogenetic relationships. The takin and muskox, like most of the Bovidae, have the same fundamental number (FN) of chromosomes, 60. The modal diploid ($2n$) of the takin is

52 (Bogart and Benirschke, 1975) while that of the muskox is 48 (Tietz and Teal, 1967). This difference does not preclude the possibility of a close relationship and could be explained by Robertsonian rearrangements (Bogart and Benirschke, 1975). High-resolution G-banding of chromosomes revealed morphological similarities and homology of banding patterns that have been used to support close relationship of the takin and muskox (Pasitschniak-Arts et al., 1994). G-band patterns can be highly conserved (Bickham, 1981), however, and without comparisons to other related species cannot be used to define the relationship between the takin and muskox.

ECOLOGY

While aspects of behavior and ecology may change with habitat (Jarman and Jarman, 1979), evidence of behavioral and ecological similarities, despite vastly different habitats, may be used to deduce relatedness. Muskoxen inhabit treeless tundra, whereas takin usually frequent steep, heavily-vegetated mountains. The remoteness and ruggedness of takin habitat have limited attempts to investigate ecology of the takin. However, because of some recent takin studies and my own field investigations, enough information is now available to compare the basic ecology of the two species.

Habitat

The most striking difference between the species is the habitats they occupy. The present natural range of muskoxen is limited to the Arctic and Subarctic, north of treeline. This environment is mostly open with vegetation close to the ground and visibility limited by topography. Muskoxen tend to utilize terrain between sea level and 700 m in elevation, although in Greenland they may venture as high as 1,500 m (Harington, 1961). Muskoxen have adapted to survive long, harsh winters in the arctic, although they also must tolerate summer temperatures > 30^{o} C (Harington, 1961). The Arctic is a dry climate and annual precipitation tends to be < 25 cm (Tener, 1965). Annual growth of a thick layer of underwool, qiviut, provides insulation against the winter cold (Tener, 1965). Muskoxen, however, are vulnerable to prolonged periods of climatic variation. Population fluctuations in Greenland have been related to long-term shifts of weather patterns (Vibe, 1967).

Takin ranges, in contrast to those of muskoxen are limited to mountainous regions in Asia at temperate latitudes between about 27° and 34° N. Within these ranges, takins are found at elevations between 1,500 and 5,200 m (Wu, 1990). Their habitat tends to be closed with visibility limited by both the dense vegetation and steep terrain. Habitat use surveys suggest takins select against using open meadows and areas above treeline (Groves, manuscript). Takins are adapted to a seasonal climate not as extreme as that of the muskox. Snow and sub-freezing temperatures are common for at least five months of the year throughout much of their range, while in summer, temperatures regularly exceed 30° C (pers. obs.). Annual rainfall ranges from 380 to 1,020 cm (Cooper, 1923). Takins do grow an underwool which presumably provides some insulation. A dead takin I examined in August had a layer of curly underwool < 5 mm long. No long term studies of takin population dynamics have been conducted to determine if they are vulnerable to climatic fluctuations and consequent changes in food availability as are muskoxen.

Social Structure

An obvious ecological similarity between the species despite different habitats is their social structure. Both species tend to live in mixed-age and sex groups, although single males are regularly seen (Bailey, 1912; Tener, 1965; Schaller et al., 1986; Ge et al., 1990). Group sizes as high as 200 for muskoxen and 300 for takins have been reported (Bailey, 1912; Tener, 1965), although sizes of < 40 are more common for both (Tener, 1965; Ge et al., 1990). Seasonal variations in group size are common in both species with groups tending to be larger in winter than summer (Schaller et al., 1986; Ge et al., 1990; Gray, 1990).

Observations of muskox group size on Banks Island were easily made due to the open habitat and high density of muskoxen (Gunn et al., 1991). I was able to observe up to 20 groups in a day, although on average, seven groups a day were observed. Each day was treated independently, so the same animals were observed repeatedly. Observations were made of 428 groups, including single males. The largest group seen was 47 animals on 2 August, 1990. The mean group size based on all observations was 8.34 animals (*S.D.* = 4.31).

Observations of takin group size were more infrequent due to the dense vegetation and the difficulty of actually seeing and counting the animals. In total, observations were made of 47 groups, including single males. The largest group of 40 animals was seen on 24 October, 1988. The mean group size based on all observations was 6.5 animals (*S.D.* = 8.24).

Group structure in both species is loose with apparent open membership which allows individuals to join or leave groups freely, except possibly during the rut (Ge et al., 1990; Gray, 1990). On Banks Island, I observed muskox groups ranging in size from 6 to 21 merge peacefully into one group and move off together. On several occasions I also witnessed larger groups of 25 to 47 split into two or three separate groups that moved off in distinct directions.

Observations of takin group dynamics were more difficult. I did observe signs of flexible group structure, however. Although I saw a group of 40 individuals, it divided into two smaller groups while I watched and they moved off in separate directions. Observations of takin feeding and bedding areas, and tracks suggested that smaller groups moving in the same direction would merge and move on together.

The takin is somewhat unusual in that among ungulates group size tends to decrease with increasing habitat density (Hirth, 1977; Jarman and Jarman, 1979) and large animals tend to live in more open habitats (Leuthold, 1977). Thus the apparent incongruency of a large-bodied ungulate living in groups in dense habitat could be interpreted as being a characteristic evolved in the past in a different environment. The similar gregarious behaviors of the takin and muskox despite different habitats could be viewed as an indication of a common heritage.

Predator Avoidance

The takin and muskox both utilize group defense as a means of avoiding predators (Tener, 1965; Wu, 1990). Predators of muskoxen include wolves (*Canis lupus*), grizzly bears (*Ursus arctos*) and polar bears (*Ursus maritimus*; Gunn and Miller, 1982; Gray, 1987). When threatened by predators, muskoxen usually will form a tight bunch with rumps pressed together and heads and horns facing outwards. Takin predators in Shaanxi, China include Asiatic black bears (*Ursus thibetanus*), Asiatic wild dogs (*Cuon alpinus*), leopards (*Panthera pardus*), and historically, tigers (*Panthera*

tigris; J. Wu, pers. comm.). There are no published reports of takins forming a defensive circle like muskoxen sometimes do, but when threatened, I observed groups run together, stand their ground and face the threat. Local people reported being charged by takins and will not approach them closely unless armed.

The horns of both sexes of both species have similar-sharp points which can be used as weapons against predators. This is different from some other Caprinae species in which the horns are highly sexually dimorphic and evolved for dominance displays rather than defense (Geist, 1966). Other Caprinae have small horns that are used for resource defense rather than group defense against predators (Geist, 1987).

In ungulates, group defense against predators is not common and is only observed among some large-bodied species with stout horns (i.e. takin, muskox, and African buffalo; Schaller, 1977; Sinclair, 1977). The occurrence of this specialized behavior in both the takin and muskox can be interpreted as evidence of a close evolutionary relationship.

Movement

Neither takins or muskoxen migrate, although both species often move seasonally within an area (Gray, 1990; Wu, 1990). Takin seasonal movement is mostly vertical, with the animals utilizing higher elevations in summer and lower in winter, presumably in response to snow and vegetation changes (Sowerby, 1928; Wu, 1990). Muskox seasonal movements also are influenced by snow and vegetation changes (Hubert, 1974; O'Brien, 1988). Any vertical movements of muskoxen tend to be opposite those of takins, as muskoxen may move to higher elevations in winter and spring to avoid deep snow (Harington, 1961).

Both species are somewhat sedentary. Many muskoxen appear to have home ranges and movement within these ranges varies over the year (Jingfors, 1980). Daily averages of movement rates for muskoxen are reported to range from 0.5 to 9.9 km a day (Smith, 1976; Jingfors, 1980). On Banks Island, I observed groups of muskoxen in the same area for up to five days. During this time these groups moved < 0. 5 km.

Takins also appear to have home ranges in that groups of animals have been observed within the same area throughout the year (Schaller et al., 1986; Ge et al., 1990). Daily movements of takins have not been ascertained due to the difficulties in

observing the animals. Takins regularly moved the length and breadth of my 35 km^2 study area. I tracked one group which moved > 7 km without stopping in about four hours. However, I also observed groups that moved < 1 km over four days and found evidence of areas where takins had fed, based on age of feces and amount of wilting of plants, over a period of at least three days.

While takin movements are not well enough understood for an in depth comparison, takins and muskoxen appear to have similar patterns of movement both on a seasonal and daily scale. This is another ecological similarity between the species, but similar patterns have been observed in other Caprinae (Geist, 1971; Schaller, 1973, 1977), and so may not be a strong argument for relationship of the takin and muskox.

Diet

Obviously the takin and muskox are exposed to different dietary selections within their respective habitats, but comparisons can be made between feeding styles and general preferences. Muskoxen in northeast Alaska consumed at least 25 of 174 plant species identified in the area (Robus, 1981). Other studies from both Alaska and Canada report at least 35 species consumed by muskoxen (Tener, 1965; O'Brien, 1988). Food preferences change seasonally and regionally and muskoxen consume graminoids as well as some shrubs and forbs (Parker, 1978; Jingfors, 1980; Robus, 1981; Biddlecomb, 1992; Klein and Bay, 1994). Takins in Sichuan, China consumed at least 138 plant species (Schaller et al., 1986) and Wu et al. (1986) reported 114 species eaten by takins throughout their range. Takins consumed 105 of the 120 plant species I identified during a feeding use survey (Groves and Wu, manuscript). Grass and grass-like plants comprised only 10% of the number of species eaten. My examination of areas where takins fed in summer and autumn suggest they are primarily browsers, an observation supported by Schaller et al. (1986).

Similarities in the feeding styles of muskoxen and takins are apparent. Despite their different habitats, both consume a wide variety of plant species and can be considered generalists that can adapt to changing food availability. Feeding style, body size and group size in ungulates tend to be inter-related (Jarman, 1974), so the similar

feeding styles of the takin and muskox may be attributed to their similar body and group sizes as well as to a shared heritage.

MITOCHONDRIAL DNA

When evidence of relationships from characteristics traditionally used for phylogenetic reconstructions is contradictory, sequences from mtDNA may provide an objective measure for comparison (Brown et al., 1982). The rate of mutation of the cytochrome *b* gene as a protein-coding gene is useful for interspecific comparisons at the family and subfamily level (Kocher et al., 1989; Irwin et al., 1991). I used sequence data from the cytochrome *b* gene to test the hypothesis that the takin and muskox are sister taxa (Groves and Shields, 1995). One species from each of the other three Caprinae tribes (Simpson, 1945) was included in the comparison: saiga (*Saiga tatarica*) from the Saigini, Chinese goral (*Nemorhaedus caudatus*) from the Rupicaprini and bighorn sheep (*Ovis canadensis*) from the Caprini. The published sequence of domestic cow (*Bos taurus*) was included as an outgroup (Irwin et al., 1991).

Comparison and analysis of these sequences indicate that the takin and muskox are not sister taxa, but that each species is more closely related to another species. The muskox appears to belong with the goral in the Rupicaprini while the takin belongs with the sheep in the Caprini. The Ovibovini proposed by Simpson (1945) does not appear to be a valid tribe.

CONVERGENCE ?

Morphological, paleontological, cytogenetic and ecological comparisons reveal similarities between the takin and muskox (Table 6 - 1) which can be viewed as supporting a close evolutionary relationship of the two species. The results of the mtDNA comparison are incongruent with the previous comparisons and suggest the two species are not sister taxa.

Incongruencies between traditional morphological and recent molecular phylogenies have been recognized and debated upon since molecular techniques have been in use (Patterson et al., 1993). Congruence between phylogenies produced by different characteristics provides strong support for that phylogeny, but there is no

Table 6 - 1. Comparison of the takin and muskox.

Characteristic		Takin	Muskox
Morphology	Size	Large: ≥ 300 kg	Large: ≥ 300 kg
	Horns	Stout, defensive hooks both sexes	Stout, defensive hooks both sexes
Chromosomes	2*n*	52	48
	FN	60	60
Paleontology		Sparse fossil record back to *B. teilhardi*, Upper Pliocene	Rich fossil record back to *Boopsis*, Lower Pliocene
Habitat		Dense	Open
Climate		Temperate	Arctic
Social Structure		Mixed age and sex groups	Mixed age and sex groups
Predator Avoidance		Group Defense	Group Defense
Movement		Non-migratory Seasonal Movement	Non-migratory Seasonal Movement
Diet		Generalist, primarily browser	Generalist, grazer and browser
mtDNA		Closer to sheep than muskox	Closer to goral than takin

general agreement on how to treat conflicting phylogenies (Hillis, 1987), although conflicts suggest that some or all of the characteristics being analyzed should be reassessed (DeSalle and Grimaldi, 1991). Some conflicts may arise when there are unequal rates of evolution among the branches being considered. Some types of phylogenetic analysis are particularly sensitive to these unequal rates (Hasegawa et al., 1991; Stewart, 1993). Further confusion can be caused by differing rates of evolution of different portions of the genome (Hillis, 1987). Phylogenetic reconstruction within the Bovidae has been complicated by the rapid radiation since bovids first appeared 20 million years ago and the subsequent diversity and wide distribution of the family (Gatesy et al., 1992). Relationships within the family have not been fully resolved. Analysis of mtDNA sequence data of the Bovini within the Bovidae also contradicted more traditional phylogenies (Miyamoto et al., 1989).

Nonetheless, data from mtDNA have been useful in establishing relationships among species (Quinn et al., 1991; Shields and Kocher, 1991; Honeycutt and Adkins, 1993; Mouchaty et al., 1995). My analysis of mtDNA of the takin and muskox strongly separates the two species, and I believe, provides a stronger phylogeny of the species than earlier comparisons. Similarities between these species should not be attributed to their common phylogeny and shared genetic heritage, but should be reassessed as a demonstration of the force of natural selection and convergent evolution in the development of similar characteristics in separate lineages.

The difficulty of distinguishing between homologous and analogous features of morphology can complicate understanding the relationship of species (Goodman et al., 1987). Large body size distinguishes the takin and muskox from all other Caprinae (Schaller, 1977). Because increased body size confers advantages such as improved ability to withstand temperature extremes, lower mass-specific metabolic rate, ability to digest lower-quality food, nutrient economy, and ability to repulse predators (Scholander et al., 1950; Kleiber, 1975; Schaller, 1977; Hudson, 1985; Feist and White, 1989; Hofmann, 1989), large body size can be expected to evolve independently in separate lineages.

The similar body size of the takin and muskox appears to be an analogous feature which is adaptive for each species in their different environments. In the Arctic, the large body size of muskoxen is adaptive for the cold climate, consuming a low-quality winter diet (White et al., 1984), and avoiding predation. In the more temperate environment takins inhabit, large body size is adaptive for consumption of food unavailable to smaller ungulates in that habitat, either because of lower quality or physical inaccessibility, as well as to avoid predation. I propose the takin and muskox each evolved a similar body size to adapt to a "large-bodied ungulate" niche in their respective environments. Other morphological and ecological similarities can be attributed to shared body size.

Gregarious behavior and predator avoidance are closely inter-related with body size. Group defense against predators is only observed among species large enough to effectively repulse predators, but is only practical if the species lives as a group. In

addition, because group members must have horns that can be used as weapons, group defense selects against highly ornamental or sexually dimorphic horns.

Feeding style and, consequently, movement patterns also are related to body size. While not an absolute relationship, larger ungulates tend to be able to consume more generalized diets than smaller ones (Jarman, 1974; Hofmann, 1989). These less-selective feeders can live in groups because there is not intense intra-group competition for food resources. A large-bodied herbivore consuming a generalist diet is not severely constrained by seasonal changes in food availability and quality, and should not need to migrate to find sufficient food resources throughout the year. The generalist feeding style also is adaptive to a sedentary pattern of daily movements since food availability should be relatively high in feeding areas.

Morphological and ecological characteristics are inter-related and evolve in response to similar types of ecological pressures, so a suite of similarities can develop between species that are not closely related. Evidence from mtDNA sequences suggests this is the case for the takin and muskox. What is surprising about these species is that the similarities have appeared in such different environments. The apparent contradictions of the takin being a large-bodied and gregarious ungulate living in dense vegetation suggests the advantages of group living to avoid predation outweigh the costs of maintaining group structure in that habitat.

SYNOPSIS

This project addressed ecological and molecular comparisons of the takin and muskox. I attempted to resolve a long-standing debate about the relationship of the species and whether their placement in the tribe Ovibovini within the Caprinae subfamily is valid.

Due to a dearth of knowledge about takin ecology, this project began with a field study of takins in Shaanxi Province, China. Extremely dense vegetation made takin observations difficult, however, data on group size in relation to habitat, habitat use and feeding preferences was collected. These data suggest takins are not particularly selective in habitat use and have a generalist feeding style. Thus, because of abundant forage throughout their home range, they use the breadth of available habitat. Takins are unusual among ungulates in that they are gregarious but live in dense habitats. This gregarious behavior appears to be an adaptation to minimize predation risks; takins often depend upon group defense to evade predators.

When the ecology of takins and muskoxen were compared, similarities were found to exist. Both species are gregarious, living in similar-sized groups of mixed-age and sex composition, both are non-migratory, consume a generalist diet and both utilize group defense to avoid predation. There is one striking difference, however, between the takin and muskox; takins inhabit dense habitats at temperate latitudes while muskoxen live in open habitats at arctic latitudes. The ecological similarities despite different habitats appear to support the hypothesis of a close relationship between these species and to substantiate earlier comparisons of the species which also espoused the relationship.

Using modern molecular techniques to generate cytochrome *b* sequences from mtDNA, the hypothesis of close relationship between the takin and muskox was definitively tested. The sequence data suggest the takin is more closely related to sheep than the muskox and the muskox is closer to the goral than the takin. Therefore, the hypothesis of close relationship was rejected and the tribe Ovibovini does not appear to be a valid classification.

If the takin and muskox are not genetically related, how can the similarities between them be explained? This study proposes that these two species are an example of convergent evolution and that each evolved independently to fill a "large-bodied ungulate" niche in their respective environments. Morphological and ecological characteristics are inter-related and evolve in response to similar types of environmental pressures, so a suite of similarities can develop between species that are not closely related. The sequence data suggest these characteristics evolve at a rate independent of mtDNA. Due to adaptive advantages, large bodies can be expected to evolve repeatedly in separate lineages as apparently has occurred with the takin and muskox. Other morphological and ecological similarities can be attributed to shared body size.

The unexpected result of the lack of close relationship between the takin and muskox led to questions about the broader picture of Caprinae phylogenetics. Due to the mountainous habitat in which most Caprinae species have evolved, they have left a sparse fossil record and their relationships are not clear. Sequences of the cytochrome *b* gene from 11 Caprinae species were compared. Apparent rapid and unequal rates of evolution among these species did not allow complete resolution of Caprinae relationships, but this broader comparison still strongly separated the takin and muskox into distinct clades.

Sequencing technology provided a new perspective on the debate surrounding relationship of the takin and muskox and served to increase the distance between what were thought to be close species. This same technique was used to investigate muskoxen more closely in an attempt to define the separation between two subspecies. On the intraspecific level, variation among muskoxen was so low, differences between subspecies could not be defined. Based on these results, there appears no reason to prevent the interbreeding of indigenous muskoxen on the Canadian mainland and introduced muskoxen expanding their range eastward out of Alaska. Apparently, a long history of repeated bottlenecks has reduced genetic variability among muskoxen to extremely low levels. This lack of variability, at present, has not impacted on survival of this species.

The logical progression of this project would next be to investigate intraspecific variability among takin subspecies to determine if they also are bottlenecked populations and if that is another similarity they share with muskoxen. The takin is well-adapted to its environment, however it faces severe threats from habitat destruction and competition with an ever-expanding human population. To better understand this species, studies must be conducted soon while intact wild populations still survive.

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